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ANATOMY AND METAMORPHOSIS OF THE APPLE MAGGOT, RHAGOLETIS POMONELLA WALSH [1]

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INTRODUCTION

Since all things of the present are products of the past, we can understand nothing rightly without knowing its history. This applies even to insects. But, among insects, so many species are so much alike that it is not necessary to know all phases of each one separately; the story of one may be repeated in the history of many others. Thus, the study of the apple maggot, here presented, explains a type of development and structure common to a large group of flies that includes the various other fruit-flies, the root-maggots, the house fly, the blow-fly, the sheep tick, and all the host of forms related to these species, embracing most of the important fly pests of man, domestic animals, and cultivated plants, as well as many beneficial species that are parasites of other insects.

The larval form of these flies is called a maggot, but the maggot has had the strangest history of all insects, and an obscure one, too, for it is known only to a few entomologists. Yet, because of the economic importance of maggots, much has been written about them and, unfortunately, many of the published descriptions are vague or inaccurate because the writers did not understand the history of their subjects.

The apple maggot, the larva of *Rhagoletis pomonella* Walsh, is an insect highly favored by nature. It lives among a superabundance of food, it grows to maturity in almost complete security from enemies, and is never exposed to the chance of being poisoned by the sprays of the orchardist. Its mother is a small fly (fig. 1, A) with a sharp drill at the end of her body by means of which she pierces the skin of the apple (fig. 1, B) and deposits her egg in the flesh beneath. The young maggot, hatching from the egg, has only to burrow into the fruit (fig. 1, C), for here it spends its days tunnelling about and feeding on the pulp (fig. 2) until it is full grown.

EXTERNAL STRUCTURE

In general appearance the apple maggot is a smooth, white, wormlike creature, 7 or 8 mm. in length when full-grown, having the external form and structure shown in Plate 1, A. Its body consists of eleven distinct segments and a small, retractile, conical end-piece in front (*LH*), which serves as a head and carries the pair of mouth hooks (*Hk*). The first body segment (*I*) bears the anterior larval spiracles (*ASp*) and the last segment (*VIII*) carries the posterior spiracles (*PSp*) and the anus (*An*).

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There has been much useless discussion in print on the subject of the number of segments in the maggot, by writers unfamiliar with the fundamental facts of its structure, though the correct morphology of the muscoid larva has been known for nearly 60 years, ever since Weismann (*53*)[2] published the results of his studies on the blow-fly. That the segment carrying the anterior spiracles (Pl. 1, A, *ASp*) is the true first body segment is proved by the fact that the stalks

FIG. 1.—The apple maggot (*Rhagoletis pomonella*): A, adult female (× 7); B, female fly puncturing the skin of an apple preparatory to depositing an egg; C, section of an apple showing an egg inserted at *a*, and a young maggot tunnelling into the pulp at *b*; D, an egg (greatly enlarged)

of the prothoracic leg buds are attached to its ventral surface (Pl. 3, F, L_1) and evaginate from it. Likewise the attachment of the other leg buds (Pl. 3, F, L_2, L_3) and the buds of the wings and halteres to the next two segments proves that these (*2* and *3*) are the mesothorax and the metathorax. The following eight segments (*I–VIII*) belong to the abdomen, but the last one is evidently a combination of the primitive eighth, ninth, and tenth segments.

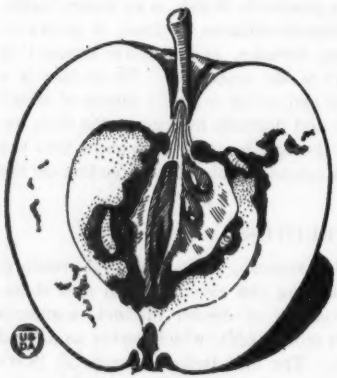

FIG. 2.—An infested apple cut open showing the tunnels of the apple maggot through the flesh

The larval head in some species of maggots is divided into several parts by transverse constrictions, one of which has been called Newport's "segment," but such rings are not true segments. Moreover, a study of the maggot's development shows that its apparent head is, at most, but a small part of the true head, and that the mouth hooks are not "mandibles" as some writers assume them to be.

The larval head (Pl. 1, A, *LH*) of the apple maggot is a simple cone, sometimes suggesting a double structure by a transverse fold which appears when the head is partially retracted. The anterior end is blunt and snoutlike (Pl. 1, B) and bears two papillæ (*g*, *h*) on each of its anterior angles. These papillæ support peglike sensory end-organs, shown more enlarged in Plate 1, C and D. On the middle of the lower surface of the head is the larval mouth (Pl. 1, B, *Mth*) from which project the two large oral hooks (*Hk*). These hooks lie in lateral pouches of the oral cavity formed by median dorsal and ventral tonguelike ridges (*k*

[2] Reference is made by number (italic) to "Literature cited," p. 33-36.

and *l*). On the lateral lips of the mouth are two pairs of chitinous teeth which appear to serve as guards against the hooks. Immediately laterad of each pair of hooks is a third sensory organ (*s*), shown more enlarged in Plate 1, E. The lips of the mouth, posterior to the teeth, are marked by ridges as shown in Plate 1, B. In some other species these ridges are more extensive and spread out like the ribs of a fan from the angles of the mouth. They perhaps serve to conduct the liquid food of the maggot into the oral cavity.

The oral hooks (Pl. 1, A, B, *Hk*) are strong organs of the mouth formed for grasping, clawing, and tearing. The apple maggot uses them for tunnelling through the fruit and for breaking down the pulp in order to liberate the juices and protoplasm of the apple cells which constitute its food. The hooks move up and down on a basal articulation, not sidewise as do mandibles, and they can be protruded or withdrawn by an evagination or retraction of the oral cavity. When the hooks are very forcibly withdrawn the head itself frequently disappears by inversion into the thorax. The mouth hooks differ characteristically in the three larval stages as shown in Plate 1, L, M, and N. In the first instar (Pl. 1, L) the second or proximal claw is well developed, in the second instar (Pl. 1, M) this claw becomes reduced, and in the third (Pl. 1, N) it disappears. The hooks are articulated to a chitinous framework in the wall of the pharynx and are worked by sets of special muscles, but those parts will be described in connection with the pharyngeal skeleton.

On the body of the maggot the intersegmental lines are marked by circular swellings (Pl. 1, A) widest on the ventral surface and most pronounced on the posterior two-thirds. All but the first of these ridges are covered by minute hooklets (Pl. 1, G) arranged in several irregular, broken rows, forming bands at the junctures of the segments. In the wider bands the hooklets of several extreme anterior and posterior rows are turned forward and backward respectively (Pl. 1, F), while those of the median rows are turned backward and forward respectively. That the band of hooklets does not mark the end of either of the adjoining segments is shown by the attachment of the muscles of the body wall (Pl. 4, C, E) along distinct lines between the median rows of hooklets in each band.

Anterior spiracles are present only in the second and third instars of the larva, and in the pupa, and they differ in each stage, since each successive pair is newly formed and not developed from those of the preceding instar. But the spiracles are always located on the prothorax and, in the larva, project like ears from the upper lateral parts of this segment close to the posterior margin (Pl. 1, A, *ASp*). Each is a lobelike structure, flattened in a vertical plane, arising from a small mound of the hypoderm. The free edge carries many small papillæ. The spiracle of the second instar (Pl. 1, H) is smaller and simpler than that of the third. In the third instar the lobe is somewhat subdivided and the papillæ are longer (Pl. 1, J). The papillæ are perforated at their ends, though ordinarily it is impossible to see the openings. But if a larva is killed in hot water and transferred immediately to cold water or to weak alcohol, the ends of the papillæ will be turgid with air, and a high-power objective will often show a slitlike aperture gaping wide in the tip of each (Pl. 1, K). Pressure on the base of the lobe will discharge tiny bubbles of air from these apertures.

The openings of the spiracle lead into a cavity within the lobe, which is prolonged backward like a stalk beneath the hypoderm to meet the end of one of the two great dorsal trunks of the tracheal system (Pl. 2, G, *Tra*). This cavity is the *stigmatic chamber* (Pl. 1, H, J, *SpC*). It is always distinguished from the adjoining trachea by its yellow color and the granular texture of its walls, and by the lack of spiral thickenings in its lining. In the second instar the stigmatic chamber of the anterior spiracle is a simple slender tube (Pl. 1, H, *SpC*); in the

third instar it is much larger and presents a clear, circular, windowlike area (Pl. 1, J, *n*) in the outer side of its base, directed outward and forward. This area is connected with the body wall (Pl. 1, I, *BW*) just ectad of the base of the external lobe by a short cuticular tube (*v*) which ends in a circular scar (*m*) on the body wall. The same structure is repeated in the prothoracic spiracle of the pupa (Pl. 2, A) and we shall see that it is characteristic also of the posterior spiracles of the second and third larval instars. It is to be explained as follows: Each spiracle is a newly formed structure developed, not within that of the preceding instar, as is usual in other cuticular structures, but from the inner side of the base of the stigmatic chamber. At each molt the old spiracle is cast off and the lining of the stigmatic chamber, together with the tracheal intima, is drawn out attached to it through the channel formed by the new cuticle of the old chamber. The latter then shrinks, as the new chamber becomes functional, and is reduced to the cuticular tube (*v*) connecting the base of the chamber with the new chitinous layer of the body wall, the closed ends forming the external and internal scars (*m* and *n*).

The successive formation of new spiracles in each instar of the fly larva has been described by De Meijere (*30*) for such a large series of genera from Mycetophila to Hydromyza that there can be no doubt that it is characteristic of all the amphipneustic Diptera. Though the form of the external spiracular lobe may vary from a simple projection to a many-fingered flap or to a long, slender tube, the fundamental structure is always the same. It has not been proved for all stages that the new stigmatic chamber is an outgrowth from the old, but Weismann (*54*) has shown in Corethra, and Wahl (*49*) in Eristalis, that the stigmatic chambers of the pupal spiracles grow out from the ends of the tracheæ as solid masses of cells, proliferated from the tracheal epithelium, in which a lumen appears later. It would seem more reasonable that the chambers should be formed as secondary ingrowth from the body wall since they have no tænidia nor any other appearance of tracheal structure, and Haake (*18*) says that they are thus formed in the larva of Trigona. De Meijere (*30*) says that the inner walls of the chamber ("filzkammer") are usually ciliated, and that in some forms they are braced by chitinous bars across the cavity. In Rhagoletis the chambers are unobstructed and their walls appear to be rugose but not ciliated.

These dorsal spiracles of both the larva and the pupa are, in any case, secondary structures. They are developed in connection with the *dorsal* longitudinal tracheal trunks (Pl. 2, G), while the primitive spiracles, temporarily closed in the maggot, belong to the *lower* lateral trunk. That special breathing structures of a similar nature occur in other orders is indicated by the description of a respiratory organ in a beetle larva, Donacia, given by Böving (*9*). Here a hollow, apparently perforate, spinelike process is situated on the back of the eighth segment over an end-chamber of a trachea, and contains a prolongation of the latter. At each molt the spine is cast off, bringing the tracheal intima with it, while a new spine is generated by the new cuticle and a new branch of the tracheal end-chamber grows out into it.

The last body segment of the maggot is the largest (Pl. 1, A, *VIII*). The posterior part of its upper surface is flat, slopes downward, and bears the posterior spiracles (*PSp*). The anus (*An*) is situated on the rear part of its ventral surface between a pair of lobes. The stigmatic surface is surrounded by eight tubercles, two above and two below the spiracles, and two very small ones on each side. A fifth pair occurs between the substigmatic pair and the anal lobes. In the pupa the eighth pair of abdominal spiracles is situated on this segment, and the part bearing the anus is differentiated as a small segment which becomes the tenth of the adult. The terminal segment of the larva, therefore, is evidently a combination of the eighth, ninth, and tenth abdominal segments.

The posterior larval spiracles (Pl. 1, A, *PSp*) are present in each of the larval instars. In the first instar each spiracle has two apertures (Pl. 2, D), in the second (Pl. 2, B) and third (Pl. 2, C) three apertures. The openings are surrounded by dark chitinous rims known as the *stigmatic plates* (Pl. 2, D, *SpPl*), which have different shapes characteristic of each instar. Beneath the plates are stigmatic chambers (*SpC*) set on the ends of the tracheæ, which are similar in structure to those of the anterior spiracles and have the same yellow color.

The stigmatic plates of the posterior spiracles of the first-instar larva are simple oval rims about each aperture, widest on the outer sides (Pl. 2, D, *SpPl*). Four groups of delicate radiating hairs arise from the cuticle at the upper, outer, and lower margins of each pair of plates. The stigmatic chambers (*SpC*) are long, tubular end-sacs of the tracheæ (*Tra*), in line with the tracheal axes.

In the second instar the posterior stigmatic plates are elongate-oval in form and each has from six to eight teeth on the inner edge of each lip guarding the aperture to the stigmatic chamber (Pl. 2, B). Four large, fan-shaped bunches of hairs arise from the cuticle around each spiracle, one above, one below, and two lateral between the outer ends of the plates. Each stigmatic chamber (*SpC*) divides distally into three lobes corresponding with the three stigmatic plates and their apertures. On the inner side of its base is a small circular scar connected with the body wall by a shrunken strand of cuticle (*v*). This, as in the case of the anterior spiracles, is the remnant of the channel through which the first stigmatic chamber and the lining of the tracheæ were drawn out at the first molt. The position of the new spiracles, however, is reversed at the two ends of the body; in front the new spiracle being formed mesad to the old.

In the third instar the apertures of the posterior spiracles are linear slits in long, slender stigmatic plates (Pl. 2, C). Each plate is a cylindrical chitinous structure at the end of a wide lobe of the stigmatic chamber, and is open to the exterior the full length of its outer surface and at each end (Pl. 2, E). The lips of the opening are fringed with many slender prongs that curve inward like the teeth of a pair of rakes turned toward each other. The structure of these spiracles is particularly conspicuous when a larva is submerged in water, for the air in the spiracles may then be seen glistening like mercury between the prongs of the slits of each plate and bulging out at the unguarded ends. Four radiating groups of hairs arise from the cuticle about the stigmatic plates, as in the second instar, but in this, the third instar, most of the hairs are branched. The stigmatic chambers (Pl. 2, C, *SpC*) are much larger than in either of the other instars, and the scars in their bases, left by the chambers of the second instar, are large and conspicuous. These spiracles do not reappear on the pupa. When the stigmatic chambers and tracheal linings are cast out at the last larval molt the holes in the body of the pupa close.

THE TRACHEAL SYSTEM

The most conspicuous features of the larval tracheal system are the two large dorsal trunks that extend between the anterior and the posterior spiracles (Pl. 2, G. *Tra*). The trunks are connected by a dorsal commissure in each segment but the first, and they give off 11 descending lateral branches. The first and the last dorsal commissures (*q* and *s*) are much larger than the others and go straight across from one longitudinal trunk to the other. The rest (*r*) are very slender tubes and each makes a long V-shaped loop into the preceding segment. The lateral branches from the dorsal trunks (Pl. 2, F) supply tracheæ to the viscera and to the body walls. All but the first two are connected below the middle of the sides by a series of longitudinal commissures (*t*). These commissures are seldom shown in drawings of the tracheæ of the maggot, but they are a very important feature of the general tracheal system; they constitute, in fact, the

ventral lateral trunk which is always connected with the primitive spiracles. In the maggot the lateral spiracles are undeveloped and the lateral tracheal trunks are reduced in size till, by comparison with the highly developed dorsal trunks, they become inconspicuous and appear to be mere commissures between the descending branches from the dorsal trunks. But the lateral spiracles appear on the puparium (Pl. 5, A) and are opened in the pupal stage by the shedding of the tracheal linings. Finally they become functional in the adult, where the lower lateral tracheal trunk regains its importance and the dorsal one is reduced again to usual size.

Perhaps the lateral spiracles are present in some form in the hypoderm of the larva, but it is impossible to see them by surface examination until the skin hardens to form the puparium. Van Rees (*47*) noted in Calliphora that the lateral stigmatic tracheæ do not penetrate the hypoderm in the larva or in the puparium, but that each ends in a small island of cells which forms a part of the outer surface of the body. These islands regenerate the epithelium of the stigmatic tracheæ and that of neighboring parts of the longitudinal trunks. Wahl (*50*) regards them as true hypodermal imaginal buds and says that they are present on the metathorax and on the first seven abdominal segments in Eristalis, as noted also by Giacomini (*12*). Van Rees (*47*) finds them on all the abdominal segments but the eighth in Calliphora. Pratt (*40*) says that there is a series of eight microscopic, rudimentary stigmata on young larvæ of *Melophagus ovinus* L., two on the thorax and six on the abdomen. In the puparium of Rhagoletis (Pl. 5, A) eight pairs of spiracles appear on the abdominal segments, but the last is very small.

It is thus evident that the two sets of spiracles in the Diptera have no relation to each other. The temporary, dorsal larval spiracles are developed in connection with the *dorsal* longitudinal tracheal trunks; the primitive spiracles belong to the *lower* lateral trunks. The fundamental tracheal system is the same in both larva and adult, but the longitudinal trunks are proportionately developed in each stage according to the spiracular system in use—the larva and pupa using the dorsal system, the adult using the ventral system.

The larvæ of the lower flies are peripneustic like the adults, and the presence of the stigmatic islands of Van Rees in the hypoderm of the larvæ of higher forms shows that even in them the lateral spiracles have not been entirely lost. Their final opening is but a restoration to primitive conditions. The tracheal system of a cecidomyiid fly larva (*Cecidomyia resinicoloides* Williams) as described and figured by Williams (*55*) consists of two large dorsal trunks extending between the anterior and the posterior spiracles, and of two slender lateral trunks connected with lateral spiracles. The anterior spiracles are on the prothorax; the posterior spiracles are on the last abdominal segment, which is the ninth. The lateral spiracles occur only on the first seven abdominal segments. The condition here strongly suggests that both respiratory systems are present and functional at the same time in this larva, and that the lateral spiracles are in an early stage of repression. The spiracle tracheæ are still well developed in the metathorax, though the spiracles of this segment are lacking.

The larva of the higher flies, then, temporarily discards the primitive lateral spiracles and develops breathing organs of its own in connection with the dorsal tracheal trunks. It acquires first a pair of spiracles at the rear end of its body, and is metapneustic in its earliest instar. Next it acquires a second pair at the anterior end of the body, and is amphipneustic in the second and third instars. The pupa retains only the anterior spiracles of the larval system, and is propneustic. The adult discards all the larval spiracles, restores the primitive breathing system of its ancestors, and is again peripneustic.

In the facts thus outlined a basis might be found for believing that the fly maggot owes its form and structure to an early but secondary aquatic life when it floated half submerged on the surface of water. In such an environment the enlarged dorsal tracheal trunks would serve as floats, the dorsal spiracles would facilitate breathing above the surface, and the closing of the lower spiracles, with the reduction of the lateral tracheal trunks, would follow as logical modifications. The maggot form is probably not necessary, or the only one possible, in the present environments of most muscoid larvæ, but certainly, once acquired, it is one well adapted to many conditions of living and gives the muscoids a wide range of possible environment with few modifications of structure.

THE IMAGINAL BUDS

The larva of insects with complete metamorphosis consists of two sets of cells, one set including all of those cells that constitute the larval organs, the other those that will form the adult organs which replace larval parts. In general, the adult cells are much smaller than the cells of the larva, and, in the larva, they are centered in small groups called *imaginal buds*, *imaginal discs*, or *histoblasts*. It is now pretty well established for the Diptera that there are four imaginal buds in each segment, and that, all together, they form four rows of discs in the hypoderm, two in line with the wings and two in line with the legs. This applies theoretically also to the head, but the condensation of the segments in this region obscures the primitive arrangement of the buds. Each bud consists of both ectodermal and mesodermal cells, the first regenerating the hypoderm and all parts derived from it, the second regenerating new muscles and perhaps other mesodermal tissues. The alimentary canal is reformed from special cells situated in its own walls.

Most of the histoblasts do not begin their regenerative growth until the last larval instar. But, in the case of appendages that have disappeared entirely in the larva, the histoblasts may take an early start, and, in the higher Diptera, some of these begin their development in the embryo. Such buds must necessarily push inwardly in their growth, and, as a consequence, they come to lie in pockets of the hypoderm, called *peripodal cavities*. In the muscoid larvæ these pockets of the leg buds form long, stalked pouches entirely closed at their outer ends (Pl. 3, F, L_1, L_2, L_3). All such organs that begin in this precocious manner must later be everted in order to complete their development. This takes place in the pupal stage.

The saclike buds of the legs and wings growing inside the body were observed in many insects long before their true nature was known. Weismann (*53*) showed that they evert to form the external appendages and gave them the name of "imaginal discs." In Corethra he noted (*54*) that they arise from the hypoderm, but he failed to see this in Calliphora.

Künckel d'Herculais (*27*) first demonstrated that the buds of external parts are always of ectodermal origin. He proposed the name "histoblast" as a term more generally appropriate than Weismann's "imaginal discs." He, moreover, enumerated 12 pairs of buds in the Syrphidae—4 pairs in the head, 6 pairs on the thorax, and 2 on the abdomen which form the genital armature.

Ganin (*11*) added the fact that there are two pairs of buds on each of the abdominal segments, and that these buds regenerate the abdominal hypoderm and its appendages wherever appendages occur. He showed also that each bud consists of both hypoderm and mesoderm.

Viallanes (*48*) likewise recorded the presence of four buds on each of the abdominal segments, and pointed out that these fall in line with those of the thorax, forming thus four rows of buds along the entire length of the body in the larva that regenerate the external parts of the adult. Kowalevsky (*25*), studying Muscoid larvæ, found a dorsal and a ventral pair of buds on each of the first

seven segments of the abdomen and corresponding pairs on the eighth surrounding the anus. These abdominal buds, he says, are mere islands of small cells among the larger cells of the larval hypoderm. They regenerate the new hypoderm of the pupa, and those of the last segment form also the new epithelium of the rectum and the rectal papillæ.

Van Rees (*47*) describes three pairs of buds on each of the abdominal segments in Calliphora, two dorsal and one ventral. The second dorsal buds, he says, are very small, however, and lie close to the others with which they soon unite when they begin to expand. Wahl (*50*) finds also three pairs on the abdominal segments of Eristalis, two dorsal and one ventral, but the two dorsal pairs widely separated and situated close to the opposite edges of the segment. This is apparently an exceptional condition. Giacomini (*12*) describes only two pairs of buds on each of the abdominal segments of *Eristalis tenax* L_4 and says that they have the form of small invaginations in the mature larva.

Pratt (*40*), discussing imaginal buds of all insects with complete metamorphosis, sees so much significance in the presence of four rows of them that he concludes that the ancestors of insects had four series of segmental appendages along the entire length of the body. Such a theory is unwarranted if we regard the buds as the regenerative centers of each segment as a whole, and not as the histoblasts, primarily, of an appendage. The presence or absence of a leg or a wing is incidental, though the invaginated form of the bud is due to the appendage which the bud is to form. Yet all appendage-forming buds are not invaginated, as has been shown by Needham (*36*) for some beetles, where the bud is a flat disc of cells which grows outward to form the appendage beneath the larval skin.

This discussion would be more in place in the section on metamorphosis were it not for the fact that an understanding of the imaginal buds in general is necessary for an understanding of the morphology of the head and pharynx of the maggot. The metamorphosis of the higher flies *begins in the embryo*. Normally metamorphosis has to do with the transformation of the larva into the adult, and, in its more primitive manifestations, the changes do not begin until the end of the last larval stage. In the Diptera, however, the reconstructive growth has started at successively earlier and earlier stages during evolution, till, in the higher forms, some of the imaginal parts now begin to develop in the embryo.

THE LARVAL PHARYNX AND THE FRONTAL SACS

The mouth of the larva opens into a large antechamber of the alimentary canal which is functionally the larval pharynx. An elaborate chitinous skeleton (Pl. 3, B) is developed in its walls and forms the dark chitinous structures that show through the skin in the anterior part of the maggot's body, and which support the oral hooks and their muscles. Most of the illustrations published in systematic and economic papers on Muscoid larvæ show only the parts of the pharyngeal skeleton that are chitinized strongly enough to be seen through the overlying muscles and body wall. Since such figures do not give the true form or structure of the plates, they have no morphological value and convey no idea of the functions of the parts.

An intelligible description of the pharynx and its skeleton must be based on a clear understanding of two well known facts in the development of the head of the maggot and of the adult fly. The first is that the head of the fly is formed largely from two sacs inverted deeply into the body from the frontal region of the larval head. The second is that, in the higher Diptera, the true head of the larva has been invaginated, not retracted, into the mouth and has carried with it the two frontal sacs and their points of origin in the hypoderm. Hence, the so-called head of the larva is really only the neck and possibly a small part of the

base of the true head. Most of the head forms the larval mouth cavity and a part of the larval pharynx.

An examination of figure 3 will show in a very simple, diagrammatic way just what has taken place in the evolution of the maggot's head and how the parts have come to be as they are. The head of the adult fly is formed by a restoration of the invaginated parts to their normal positions, and by the development of the mouth appendages from buds in their proper morphological places. The reader should note here that the usual diagrams of the metamorphosis of the muscoid head, taken from Korscheldt and Heider (*22*), are incorrect in that they represent the frontal sacs as arising from the rear end of the larval pharynx and not from its anterior end. Such a scheme leaves nothing to account for the anterior parts of the head invaginated in advance of the basis of the frontal sacs.

The structure of the pharynx and its appendages in the larva of Rhagoletis is shown in Plate 3, C. The pharynx itself is a wide passageway from the mouth,

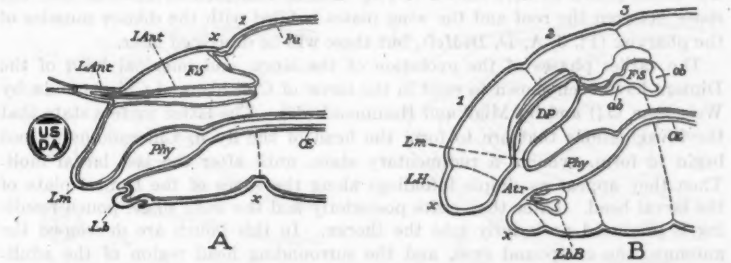

FIG. 3.—Showing the changes that have taken place in the head of a fly larva during evolution from the primitive condition of lower forms (A) to the complex structure of higher forms (B). A, diagrammatic lengthwise section of head and prothorax of larva of a lower fly, such as Chironomus, with external mouth parts (*Lm*, *Lb*) and with a small larval antenna (*LAnt*), but with a long imaginal antenna (*IAnt*) growing within a frontal sac (*FS*) or deep invagination of the hypoderm of the forming pupa (*Pu*) and with only its tip in the larval antenna; the pharynx (*Phy*) and œsophagus in normal position. B, a corresponding section through head and thorax of the maggot of a higher fly. The larval appendages are gone and the head has been turned in to form a new cavity, the atrium (*Atr*) in front of the pharynx (*Phy*), with a secondary evagination from its roof forming the bilobed dorsal pouch (*DP*) carrying the frontal sacs (*FS*) at the inner ends of its wings. The frontal sacs contain the imaginal rudiments or buds of the antennæ (*ab*) and the compound eyes (*eb*). The labrum (*Lm*) is now buried behind the root of the dorsal pouch of the atrium, and the buds of the imaginal labium (*LbB*) arise beneath the floor of the atrium. The lips of the new mouth (*x*, *x*) are derived from the back of the original head (*A*, *xx*) and the larval head (*LH*) must be the primitive neck region. (Cf. Pl. 3, F.)

between the oral hooks (*Hk*), to the oesophagus (*OE*). Its floor (*VPhy*) is a broad, convex membrane having a ribbed appearance externally (*d*) due to lengthwise ridges on its inner surface (Pl. 3, D, *d*). The lateral walls are strengthened on each side by two plates, a small triangular one (Pl. 3, C, *A*) in front and a much larger one (*B*) behind. The first plate supports the oral hook of the same side by a loose articulation at its anterior angle. Its posterior angle is more or less fused with the second plate, and its ventral angle is connected by a transverse bridge (*e*) in the floor of the pharynx with the corresponding plate of the opposite side (Pl. 3, E, *e*). A small rod from its upper edge (Pl. 3, C, *a*) helps support the roof of the pharynx, which is further strengthened by a bridge plate (*D*) between the anterior ends of the triangular plates (*A*).

The anterior part of the pharyngeal roof is convex and covers an anterior section of the pharyngeal cavity called the *atrium* by Wahl (*51*), shown diagrammatically (*Atr*) in figure 3, B. From the posterior end of the atrium the roof of the pharynx is produced upward as a dorsal pouch (Pl. 3, C, *DP*) having a

semicircular base reaching back on the sides through the first third of the lateral plates (*B*). This pouch soon divides into two wings which are produced backward nearly as far as the end of the pharynx, and are then prolonged into two long, stalked sacs (Pl. 3, A, E; fig. 3, B, *FS*). The inner surfaces of the outer walls of the two divisions of the dorsal pouch are chitinized and form the two dorsal wing-plates (Pl. 3, C, *C*) of the pharyngeal skeleton. These wings are supported by wide stalks from the upper edges of the posterior lateral plates (*B*), developed in the lateral parts of the semicircular base of the dorsal pouch (*DP*), and they are united with each other dorsally at their anterior ends by a narrow bridge in the upper part of the basal undivided part of the pouch. The stalked bags (*FS*) continued backward from the ends of the pouches are the *frontal sacs* which contain the buds of the imaginal antennæ and compound eyes.

Plate 3, D, shows a transverse section of the pharynx through the stalks of the wing plates. The roof of the pharynx is deeply concave behind the base of the dorsal pouch as here shown (*DPhy*) and as indicated in Plate 3, C. The space between the roof and the wing plates is filled with the dilator muscles of the pharynx (Pl. 3, A, D, *DiMcl*), but these will be described later.

The earlier phases of the evolution of the larval and imaginal head of the Diptera have been shown to exist in the larvæ of Culicidæ and Chironomidæ by Weismann (*54*) and by Miall and Hammond (*33*). The latter writers state that the invaginations that are to form the head of the fly in Chironomus do not begin to form, even in a rudimentary state, until after the last larval molt. Then they appear as simple infoldings along the edges of the frontal plate of the larval head. Later they unite posteriorly and the wide single pouch resulting is produced posteriorly into the thorax. In this pouch are developed the antennæ, the compound eyes, and the surrounding head region of the adult. The eversion of the parts can be observed when the larva changes to a pupa. According to Miall and Hammond (*34*) the earliest developmental stage of the frontal sacs in Chironomus represents the maximum attained in Corethra, while the sacs are still more primitive in a mosquito larva.

In the Muscoidea and Hippoboscidae the frontal sacs reach their highest development, but Pratt (*41*) has shown that in Melophagus they still originate in a pair of simple thickenings in the hypoderm on the dorsal wall of the head in the embryo. In Eristalis and Calliphora as shown by Wahl (*49, 51*), the sacs arise from a single median bud, but it seems probable that an earlier paired stage is here eliminated, for the two rudiments in Melophagus soon unite.

The paper by Pratt (*41*) on the history of the imaginal buds in *Melophagus ovinus* is the most important work we have on the embryology of the larval head in the Diptera. Our knowledge of the subject began with the work of Weismann (*53, 54*), who discovered that the head of the larva is almost completely invaginated into the body, and that the head of the adult is formed by an eversion of concealed parts. But Weismann was mistaken in believing that the sacs in which the adult head is formed are produced by the involution of the larval head. He did not distinguish the two processes, already described in this paper, namely, the formation of the frontal sacs containing the imaginal buds of the antennæ and eyes, and the withdrawing of these sacs into the body by the second process of the involution of the larval head.

Pratt (*41*) shows that the cephalic buds of Melophagus appear at a very early stage in the embryo, before the lateral mesodermic bands have extended to the dorsal side, and before the endoderm has enveloped the yolk on the ventral side. They consist at first of a pair of simple thickenings of the hypoderm on the external dorsal surface of the head just behind the stomodeum. The latter is dorsal at this stage but soon moves forward, followed by the buds, which now begin to

invaginate, forming deep crescentic slits immediately ectad to the rudiments of the cerebral ganglia. Next, the slits approach the midline where their outer ends unite to form a single, median slit, while their inner ends remain separate and increase much in size and depth. Thus is established the Y-shaped form, which is retained with subsequent modifications throughout the larval life. This stage is shown in figure 4, A, where only the unpaired basal part of the frontal sacs (*FS*) appears in a median section.

After the union of the frontal sacs in Melophagus, according to Pratt's account, the involution of the head takes place. The lips of the mouth are rolled inward, closely followed by the common base of the frontal sacs which are thus carried forward, downward, and finally posteriorly into the new mouth along with the walls of the head (fig. 4, B). There is thus established not only a new mouth, but also a new section of the digestive tract. The latter becomes the anterior part or atrium (*Atr*) of the larval pharynx. The frontal sacs (*FS*), now arising from the rear part of the newly established atrium, grow rapidly and soon attain the form they have in the young larva.

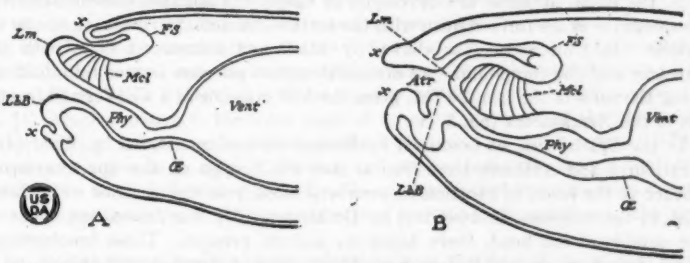

FIG. 4.—Diagrams of the development of the larval head parts in *Melophagus ovinus* (from Pratt, but relettered with parts omitted). A, a young embryo with head still in normal position, the frontal sac (*FS*) opening on frontal surface behind the labrum (*Lm*). *FS*, frontal sac; *LbB*, labial bud; *Lm*, labrum; *Mcl*, labial muscles; *Œ*, œsophagus; *Phy*, pharynx; *Vent*, ventriculus; *x*, *x*-external fold of hypoderm at base of head. B, an older embryo after the involution of the head. The new mouth is established by the folds *x*, *x*, the atrium (*Atr*) by the walls of the head invaginated in front of the folds, carrying to the interior the frontal sac *FS* and the labial buds (*LbB*) and burying the labrum (*Lm*) and its muscles (*Mcl*) deep in the body. The labral muscles thus become the dilators of the pharynx. Comparing these figures with those of figure 3 shows a complete parallelism between this individual embryonic development and the evident phylogenetic modification of the dipteran maggot

The more recent paper by Wahl (*51*) on the formation of the head in the cyclorrhaphous Diptera contains an exhaustive account of the head development as it takes place in Calliphora. In the blow-fly and in other species studied by Wahl (Eristalis, Allium) the frontal sacs originate in a single median evagination from the anterior part of the larval pharynx, forming a suprapharyngeal cavity, from which the two sacs extend backward into the thorax. Probably an earlier stage that would show the separate origin of the cephalic sacs is omitted in these species.

Pratt believed that the larval pharynx (fig. 4, B, *Phy*) results from the invagination of the larval head. Wahl showed that the larval head forms only the anterior part of the pharynx, the part which he calls the *head atrium* and from which arises the common base of the frontal sacs (*FS*).

It would seem that there must be some morphological difference between the dorsal pouch of the atrium (fig. 3, B, *DP*), in the branches of which are developed the wing plates of the pharyngeal skeleton (Pl. 3, C), and the true frontal sacs (*FS*), which contain the buds of the imaginal eyes and antennæ. The chitinous wing plates of the pharynx are shed, as are all other cuticular parts, with each

molt of the larva, but the linings of the frontal sacs are not affected by the molts any more than are the linings of the peripodal sacs of the leg and wing buds. This indicates that the dorsal pouch of the atrium is a secondary invagination, and that its wings are not parts of the true frontal sacs which contain the buds of the antennæ and eyes. This idea is suggested also by Pratt's finding that the median root of the sacs is formed after the invagination of the original buds. Both the median slit and the subsequent prolongations from it that carry the original bud sacs inward are apparently, then, to be regarded as the rudiments of the dorsal pouch of the atrium in which the wing plates of the larval pharynx are later developed.

The evolution of the frontal sacs and the involution of the larval head may be traced through the various groups of the Diptera. In the simplest forms, such as Simulium and Bibiocephala, studied by Kellogg (*19*), the head and mouth parts of the pupa are formed exactly within the corresponding parts of the larva, except that the larger antennæ of the pupa are forced to back up under the larval cuticle to find space for their growth. In Corethra, as described by Weismann (*54*), the pupal antennæ are developed in deeply invaginated pouches, but the compound eyes are formed flush with the surface, immediately beneath the larval cuticle. In Chironomus, as shown by Miall and Hammond (*34*), both the antennæ and the compound eyes are developed in pouches formed by infoldings along the sides of the front. This gives the first example of a well-formed frontal sac in the Nematocera (fig. 3, A).

In the Brachycera, as described by Becker (*3*) and mentioned by Wahl (*51*), Stratiomys and Antherix have frontal sacs which open on the upper, external surface of the head, in Stratiomys even well back from the anterior end. Likewise, in Lonchoptera, as described by De Meijere (*31*), the frontal sac opens on the outside of the head, there being no atrium present. These brachycerous forms, therefore, present the same condition of head development as is found in the Nematocera, and De Meijere designates them as the "Anatria." In Corethra (Weismann) and Chironomus (Miall and Hammond) the imaginal buds, including those of the head, are not formed till the last larval stage. The time of their origin in other Orthorrhapha is not known.

It is only in the Cyclorrhapha, so far as known at present, that the frontal sacs become drawn into the interior of the body by the involution of the larval head, with the consequent formation of a new anterior section of the larval pharynx, the head atrium. Such forms are the "Atriata" of De Meijere. In the higher families of this group (Muscidae, Hippoboscidae) all the imaginal buds begin their development in the embryo, suggesting that the high degree of development they have attained has forced them to this precocious start.

Thus we see that, as complicated as the head structure may be in the larvæ of the higher flies, it has reached this extreme phase by a gradual evolution from a simple condition such as that which prevails in the Neuroptera, where all pupal parts are formed inside the corresponding larval parts, as illustrated by Kellogg (*19*) in studies of *Corydalis cornuta L.*

Among the Orthorrhapha the head problem is further complicated by the fact that the larvæ show also various degrees of head retraction. Holmgren (*14*) and Becker (*3*) have attempted to explain on this basis the modifications in the Cyclorrhapha. But the structure of the parts in this group can not be interpreted as the results of a retraction of the larval head, and the idea has been refuted by all who have studied the embryonic development. Still, while the alteration of the head is described as an "involution," Pratt (*41*) has shown that what really takes place in the embryo of Melophagus is a forward growth of a circular fold of the hypoderm from the rear part of the head (fig. 3, A, *x*, *x*) which eventually forms the lips of the new mouth (fig. 3, B, *x*, *x*). Processes of growth should probably not be described by such mechanical terms as either

"retraction" or "involution," since they are due to different rates of growth in neighboring parts; but the results in the case of the head of the maggot are equivalent to an involution of the head of the embryo.

The frontal sacs of the mature larva of Rhagoletis (Pl. 3, A, E, F, *FS*) are long-stalked pouches extending backward from the ends of the pharnygeal wings (*C*) into the metathorax where they lie against the anterior surfaces of the retracted brain lobes (Pl. 3, F, *Br*). At this stage each sac is distinctly differentiated into two parts, a terminal transverse enlargement containing the bud of the compound eye (*ob*), and a swelling of the stalk which contains the bud of the antenna (*ab*). The buds are thickenings of the walls of the sacs produced into their lumina. Each optic bud receives a nerve from beneath the brain lobe of the same side.

In the embryo of the sheep tick, *Melophagus ovinus*, as described by Pratt (*41*), a tonguelike lobe filled with vertical muscle fibers projects above the mouth and in front of the origin of the frontal sacs. This lobe is evidently the labrum (fig. 4, A, *Lm*). During the involution of the head it is drawn into the mouth, along with the other parts, and eventually comes to form the roof of the anterior part of the larval pharynx, just posterior to the root of the frontal sacs (fig. 4, B). Its muscles (*Mcl*) now stretch between the roof of the pharynx and the anterior parts of the frontal sacs, and apparently become the pharyngeal dilators of the larva (Pl. 3, A, D, F, *DiM cl*). These muscles consist of two lateral masses (Pl. 3, D) attached above to the inner faces of the wing plates (*C*) of the pharyngeal skeleton, and below to the flexible, invaginated roof of the pharynx (*DPhy*). Their anterior ends lie against the rear wall of the transverse base of the dorsal pouch (Pl. 3, C, *DP*) of the atrium. The contraction of these muscles and the antagonistic flexibility of the pharyngeal roof give the sucking action to the larval pharynx.

A ventral pair of imaginal bud sacs arises from the floor of the atrium in the larva (fig. 3, B; Pl. 3, A, F, *LbB*), which are evidently the buds that are to form the proboscis and the lower parts of the head of the adult. In the embryo of Melophagus, according to Pratt (*41*), these buds arise in the normal external position ventrad to the mouth (fig. 4, A), and are withdrawn into the atrium by the subsequent involution of the larval head (fig. 4, B). Giacomini (*12*) describes the labial buds of *Eristalis tenax* as arising on each side of the opening of the salivary duct.

It is evident, now, that if the fly were to have mandibles, buds of these appendages should be found somewhere at the inner end of the atrium. But no cyclorrhaphus species possesses mandibles in the adult stage, and no mandible buds have been noted in the larvæ.[2] The oral hooks of the maggot have often been called "mandibles," but if they were true mandibular appendages, they should have been carried into the mouth in advance of the root of the dorsal pouch of the atrium and the bases of the ventral buds.[3] Instead, they arise from a region that is derived either from the original neck or the extreme base of the head. The structure and musculature, too, of the hooks is entirely nonmandibular. They are solid chitinous organs shed with each molt of the larva and are not renewed by the pupa. They arise by wide vertical bases from the cuticle at the inner ends of the lateral pockets of the mouth (Pl. 1, B), where each loosely articulates by a single median knob on its base with the anterior end of the triangular plate (Pl. 3, C, *A*) of the pharyngeal skeleton. The motion of the hooks is vertical and both work together, though each has its own set of extensor and flexor muscles (Pl. 3, F). Since nearly all the muscles of the maggot degenerate in the pupal stage, the presence of well-developed, special larval muscles for the oral hooks is not evidence that the hooks are derived from primitive

[3] Regarded as parts of maxillæ by de Meijere (Zool. Jahrb., Sysb., Bd. 40, 1915-17, p. 177-322).

structures. They are evidently secondary movable appendages of the cuticle brought forward by the involution process from the back of the head or the neck to the present position at the sides of the new larval mouth.

A recent paper by Bishoff (*7*) reopens the question of the morphology of the oral hooks in the Cyclorrhapha, but gives no convincing evidence of suggested homologies. Finding some fly larvæ, an unidentified species living in mushrooms, having *four* instead of two mouth hooks, Bischoff presents their case as one of a maggot having both mandibles and maxillæ. He gives no discussion to substantiate such an identification, though he explains at some length that the specimens are not in a molting condition and that neither pair of hooks can, therefore, be explained as those about to be cast off with the slough. Next, he discovers a species having only two oral hooks and a pharyngeal skeleton similar to that of his four-hooked species. Such a combination he finds in Coenosia. Then, by comparison, he decides that the single pair of Coenosia represent the inner hooks of the other species, and hence the *mandibles*. The weakness of the argument lies in the assumption that any larval mouth hooks are ever either mandibles or maxillæ—the very thing of which we lack the proof. Bischoff makes no reference to the work of Pratt (*41*) or any other on the actual development of the head and the mouth parts. He is cautious, however, about extending his conclusions to all the Cyclorrhapha, and promises a special paper on the cephalopharyngeal skeleton of this group.

In first-instar Muscoid larvæ there is a small, median, chitinous tooth in the roof of the atrium, which some writers have suggested might be the true mandibles fused into a single rudimentary appendage. The fact, however, that the tooth is situated before the root of the dorsal pouch shows that it, too, belongs to the rear part of the head. The tooth is shed with the first larval molt and does not reappear.

The upper edge of each lateral plate of the pharynx (Pl. 3, C, *B*) and the lower edge of each wing plate (*C*) are deeply incised parallel with their margins. On the lateral plate a cartilagelike ridge (*b*) arises in the marginal notch and turns obliquely downward across the outer surface of the plate. On the wing plate a similar ridge extends from the notch around the posterior edge of the plate, forming a prominent marginal thickening (*c*). These ridges serve for the attachment of muscles (Pl. 3, F). The posterior third of each lateral plate and the posterior and dorsal parts of each wing plate are but weakly chitinized and fade off into transparency.

The external musculature of the pharynx is very simple, consisting of four sets of muscles. These are the dorsal protractors (Pl. 3, F, *DPMcl*), the lateral protractors (*LPMcl*), the extensors of the oral hooks (*EMcl*), and the flexors of the oral hooks (*FMcl*). The dorsal protractors are inserted on the posterior surfaces of the wing plates (*C*) and along the dorsal edges of their marginal ridges (*c*). Each arises along the mid-dorsal line of the prothoracic segment (*1*) in a row of fingerlike divisions that criss-cross with those of the opposite side. The lateral protractors (*LPMcl*) are inserted along the ventral edge of the marginal ridge (*c*) of each wing plate, and go forward, downward, and outward to the latero-ventral walls of the prothorax, as indicated by the arrow in Plate 4, C. The protractors pull the entire pharyngeal structure forward, thus everting the mouth and fully exposing the oral hooks. There are no specific retractors of the pharynx, its retraction being accomplished by the general contraction of the muscles of the body wall (Pl. 4 C). The lateral and ventral muscles of the first body segment are inserted anteriorly around the base of the larval head, but six slender dorsal muscles are inserted on the membranous roof of the atrium between the prongs of the triangular plates (Pl. 3, C, *a*). The motions of a live

maggot suggest that the anterior parts are extended mostly by blood pressure resulting from the constriction of the rest of the body and the retraction of the rear parts; but when the head and prothorax are fully everted the oral hooks can be still farther protruded by the pharyngeal protractors.

The independent up-and-down motion of the oral hooks is accomplished by their special extensor and flexor muscles (Pl. 3, F, *EMcl*, *FMcl*), which lie against the sides of the lateral plates of the pharynx (*B*), beneath the lateral pharyngeal protractors (*LPMcl*). The two on each side arise posteriorly from the cartilage-like ridge (*b*) of the lateral plate (*B*), and are inserted by narrow tendonlike ends on the upper and lower lobes, respectively, of the wide bases of the hooks.

At the end of the third larval instar, when the hardened puparial skin is separating from the body of the insect within, the hypoderm and new cuticle about the pharyngeal skeleton are loosened and form a sheath over all the external parts of the pharynx and upon the inner faces of the wing plates and the upper surface of the pharyngeal roof. The muscles are thus detached from their skeletal supports. The dilators are retracted from the rear wall of the base of the dorsal pouch (Pl. 3, C, *DP*), being now contained in a tongue of the body cavity lying like a plug loosely in the space between the wing plates and the roof of the pharynx. In this way the cuticular parts are prepared for the final molt, though they remain in place until the second stage of the pupa, when the pharyngeal skeleton and the oral hooks are finally cast out along with the lining of the œsophagus.

All the pharyngeal muscles go into histolysis during the early period of metamorphosis. The protractors and the muscles of the oral hooks apparently have no homologues in the adult, but the dilators may be prototypes of those of the fly.

THE ALIMENTARY CANAL AND SALIVARY GLANDS

The alimentary canal and its convolutions are shown in Plate 4, A. The narrow œsophagus (Œ) proceeds from the rear end of the pharynx backward between the frontal sacs (*FS*) and the brain lobes (Pl. 3, F, *Br*) to the globular proventriculus (*Pvent*) in the first abdominal segment. The ventriculus (*Vent*), immediately following, has four short gastric caeca (*GC*) on its anterior end, and is disposed in many loops and coils from the first to the seventh abdominal segments, forming most of the length of the alimentary canal. Its final loop meets the intestine at about the middle of the body. The latter makes first one or two coils and then proceeds as a straight tube (*Rect*) to the anus along the left side of the other viscera.

The salivary glands are two thick cylindrical tubes lying latero-ventrally in the front part of the body cavity (Pl. 3, F, Pl. 4, A, *SalGl*). Their ducts unite into a median one (Pl. 3, F, *SalD*) which opens on the floor of the mouth in front of the ventral bridge (Pl. 3, C, *e*) of the anterior lateral plate (*A*) of the pharynx and between the imaginal buds of the labium (Pl. 3, F, *LbB*).

The four Malpighian tubules (Pl. 4, A, *Mal*) arise in pairs from two short basal tubes that originate in small ampullæ on the anterior end of the intestine (Pl. 4, B). In the adult the basal ampullæ of the tubules disappear (Pl. 4, B_1).

In old larvæ the terminal parts of the Malpighian tubules are commonly much enlarged, often to twice their usual diameter, and are so prominent by their size and opaque white color that they show through the body wall of a live maggot as conspicuous long white bodies in the rear half of the abdomen. This condition of the tubules is due to a great mass of transparent crystals contained in the lumina of the swollen parts. The crystals are structureless bodies varying in size from minute spherical grains to large irregularly oval or ovate stones, 40 microns in diameter (fig. 5, A).

The clear parts of the tubules usually contain only a small amount of minutely granular matter, but occasionally parts of them are filled with crystals of the form shown in figure 5, B. The larger of these, which reach a diameter also of, about 40 microns, are clearly concretions about a central body. Wherever they occur the epithelium of the tubule is much reduced.

THE FAT BODY

The fat tissue of the apple maggot occupies much space in the body cavity, and gives the larva its opaque white appearance. Two large masses of fat cells, however, in the anterior part of the body are particularly conspicuous on account of their pale yellow color. These arise above the salivary glands on each side and meet along the middle of the back. The rest of the tissue forms loose, white masses of a delicate texture, lying along the sides of the body, among the loops of the alimentary canal, and on the floor of the abdominal segments.

The fat cells are very small in young larvæ, and at first contain but little fat. During the third larval stage, however, they increase rapidly in size and their cytoplasm becomes filled with large oily globules. By the middle of this stage the fat cells are mostly from 43 to 46 microns in diameter (fig. 6, A, B), and, at the end of it, they have increased to more than twice this size, being now from 100 to 120 microns in diameter, and are so charged with fat that, in surface view (C), they appear to be mere spheres of oil globules with a surface film of granular protoplasm. When the maggot is contracting to form the puparium the fat cells reach their maximum size, 200 microns or more in longest diameter (D), while the cytoplasm is reduced to branching strands between the voluminous oily inclusions. The granular appearance of the cytoplasm has become more pronounced.

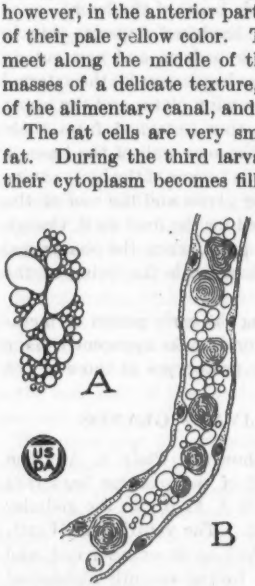

FIG. 5.—A, crystals from the terminal enlarged parts of the larval Malpighian tubules; B, crystals occasionally found in other parts of the tubules, the largest 40 microns in diameter

It has been asserted by some writers that the great vacuoles in the fat cells at this stage do not contain fat. Berlese (*4*) has stated that this is true of the fat cells of the blow-fly, and Koehler (*20*) says the same for those of the honeybee. Pérez (*39*), however, claims that the vacuoles in the fat cells of the blow-fly always contain fat, and Bishop (*8*) says the same of the fat cells of the honeybee. The present writer found that tests with Soudan III and osmic acid on the fat cells of both the honeybee and the apple maggot give very positive reactions in favor of fat in the vacuoles at all stages.

Much of the fat in the fat body of insects with complete metamorphosis disappears during the early part of the pupal period. But it is now known that the fat body of such insects is an organ of much greater physiological activity and importance than its name would imply. Though its cells form mostly fat during the larval stage, which crowds their cytoplasm with oily vacuoles, they store up also glycogen, in some species, and they elaborate proteid or albuminoid bodies which finally accumulate in large numbers in the cytoplasm. For this reason Berlese (*4*) has appropriately called the fat cells *trophocytes*.

The presence of glycogen in insects has not been generally noted, but Bataillon and Couvreur (*2*) have shown for the silkworm, and Straus (*44*) for the honeybee, that the larva stores up glycogen in increasing amount till the end of its life, and that this glycogen is then rapidly consumed during the pupal stage. These investigators, however, did not attempt to locate the glycogen in the body of the

insect; but the fat cells of a mature honeybee larva stain rapidly in iodin and retain a rich, red-brown color after long washing in alcohol. This is the common test for glycogen. The same test gives weaker results on the fat body of a tent caterpillar (Malacosoma), and no reaction at all on that of the apple maggot. No one, however, has yet shown by chemical analysis of any fly larva whether it contains glycogen or not.

The accumulation of proteid or albuminoid bodies in the fat cells was described by Berlese (*4*) for the blow-fly, and has now been noted by all subsequent observers, though the usual test has been that the bodies in question are colored by eosin and other protoplastic stains. The writer, however, applied the test with Millon's fluid to the fat cells of the honeybee pupa and obtained the proteid reaction with characteristic pink color in the non-fatty inclusions of the cytoplasm.

The fat body, then, is a very important organ in insects with complete metamorphosis, its functions being necessary to the proper reconstruction of the imago, and we can readily understand why an insufficiency of food or an improper diet during the larval period may result in a delay of the time of pupation, in the production of undersized adults, or in the death of the insect during its attempt at transformation.

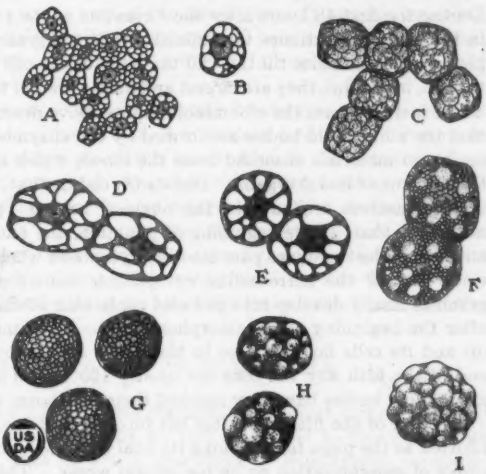

FIG. 6.—Fat cells of the larva and pupa. A, fat cells of half-grown larva of third instar, seen in optical section, the cytoplasm filled with oil droplets; 43–64 microns in diameter. B, a single cell of the same, more enlarged. C, surface view of fat cells of mature larva, showing oil globules crowded beneath surface film of granular protoplasm; 100–120 microns in diameter. D, optical section of fat cells from larva contracting to puparium; 215 microns in longest diameter. E, optical section of fat cells just after formation of puparium, the cytoplasm now coarsely granular with proteid contents, oil less abundant. F, optical section of fat cells 48 hours after formation of puparium, the oil contents greatly reduced, the proteid granules increased in size and numbers; 135 microns in diameter. G, free spherical fat cells of fifth day (115 hours) filled with large proteid grains; 130–150 microns in diameter. H, surface view of fat cells at end of 14 days, proteid contents decreasing, fat increasing in globules that swell out at the surface; 150 microns in diameter. I, surface view of fat cell at end of 18 days, cell full of fat globules bulging out on all sides, in small amount of finely granular cytoplasm; 172–215 microns in diameter

Some writers have noted, moreover, that, while metamorphosis is in progress, some of the fat cells appear to act as substitutes for the Malpighian tubules when the latter are undergoing reconstruction, for at this period, according to Pérez (*39*), some of them frequently contain crystals that later disappear. In the honeybee and other Hymenoptera, some of the cells of the fat body are apparently specialized as excretory organs, since they regularly contain small crystalline bodies in their cytoplasm. No such crystalline deposits were noted in any of the fat cells of Rhagoletis at any stage.

Other cells, known as *œnocytes*, are usually found associated with the fat cells in insects, and are often regarded also as excretory in function. But the writer did not find such cells in the apple maggot, though they might show in histo-

logical sections. Giacomini (*12*) describes those of the larva of *Eristalis tenax* as conspicuous cells, arranged in groups along the sides of the abdomen, near the rudiments of the stigmata.

With the beginning of metamorphosis the fat cells begin to decrease in size. When the apple maggot has attained the puparial condition, the oil globules are less voluminous and the cytoplasmic strands are proportionately thicker (fig. 6,E). Evidently the accumulation of fat reserves has passed its peak and consumption has begun. It is to be noted, however, that the granulations in the cytoplasm have become more conspicuous and have clearly grown to be small grains suspended in the plasma.

The further history of the fat cells takes us into the period of metamorphosis. During the first 48 hours after the formation of the puparium the amount of oil in the fat cells continues to diminish, while the granular inclusions of the cytoplasm increase in size till they fill the body of the cell from nucleus to periphery, though, in general, they are largest and most crowded toward the center (fig. 6, F). These inclusions are the albuminoid grains above described. Berlese (*4*) thought that the albuminoid bodies are formed by cell enzymes given off from the nucleus acting on materials absorbed from the blood, which is now highly charged with the detritus of larval organs. Bishop (*8*) claims that, in the honeybee, the granules themselves originate in the nucleus, having "presumably the nature of nucleoli"; that, at the beginning of pupation, the nuclear membrane disappears and the nuclear granules pass into the cytoplasm where, by "progressive absorption, both of the surrounding cytoplasmic matrix and of its vacuoles, these granules finally develop into globules containing albuminoid material." Shortly after the beginning of metamorphosis in the apple maggot the fat body breaks up and its cells float off free in the blood and become spherical (G). At the end of the fifth day the cells are mostly 130 to 150 microns in diameter. The albuminoid bodies have now reached their maximum development.

The end of the fifth day after the formation of the puparium marks the time allotted to the pupa for attaining its final external form, though the internal processes of reorganization go on for several weeks. The fat cells themselves enter a period of degeneration. Their shapes become less regular, their proteid grains decrease in size and in numbers, while fat globules reappear in the cytoplasm. But the globules now bulge at the surface as if the cell wall had become too delicate to retain them (fig. 6, H). By the eighteenth day the fat cells are again reduced to masses of oily spheres (fig. 6, I), the droplets swelling out so tensely everywhere that the cell walls rupture under the slightest pressure and allow the cell contents to be scattered in the blood. Remnants of the albuminoid bodies still persist, but they form only a sprinkling of small granules in the scanty cytoplasm.

At last the fat cells break up in the blood by what appears to be a natural dissolution of their membranes. Their contents are disseminated, and the blood plasma becomes filled with oily droplets and masses of the proteid grains loosely held in protoplasmic fragments. At least, the fresh blood of specimens handled in the most careful manner is always highly charged with these elements of broken fat cells.

Nearly all investigators have noted this apparent disintegration of the fat cells during the later part of the pupal period, but some insist that it is due to the preparation or handling of the specimens studied that the fat cells normally remain intact and gradually lose their contents by osmosis through their membranes. Some claim also that most of the fat cells finally fall prey to phagocytes, and that the remainder persist to regenerate the fat tissue of the imago. Scarcely any two writers agree in all details as to the fate of the larval fat cells or the origin of the imaginal cells. The reader may find the various interpretations of observed phenomena in the works of Kowalevsky (*24*), Van Rees (*47*), Koschev-

nikov (*23*), Berlese (*4, 5*), Terre (*45*), Anglas (*1*), and Pérez (*38, 39*). The writer, in studying the metamorphosis of the fat body of the honeybee, found no evidence of phagocytosis, but was convinced that most of the fat cells do disintegrate, as described by Bishop (*8*), after a normal dissolution of their membranes. He finds also no suggestion of phagocytosis of the fat body in the apple maggot, but in all cases a disintegration of large numbers of the cells, and this in the blood of fresh specimens removed with utmost care from the puparial shell.

The origin of the fat body of the imago is difficult to determine in Diptera and Hymenoptera, but it appears most probable that it is formed from persisting larval cells, as Bishop (*8*) concludes in his study of the honeybee. Kreuscher (*26*) says that in Dytiscus the greater part of the larval fat body appears to go over without change into the adult, for the newly emerged beetle often has a fat body with cells rich in fat and full of albuminoid granules, in large part resembling the fat body of the mature larva.

However, by whatever means the larval fat cells finally give up their contents, and whatever may be their ultimate fate, it is evident that they play very important rôles in the metamorphosis of the insect, first, in the capacity of preparers of foodstuffs in the larva and of carriers of the same from larva to pupa, and, second, in that of elaborators of these reserves into food materials for the growing tissues of the adult. In the first case they use the pabulum absorbed from the alimentary canal of the larva; in the second they use the fatty reserves stored in them, perhaps also taking other materials from the blood, formed from the histolysis of the larval organs, and possibly also consuming some of their own protoplasm.

THE MUSCLES OF THE BODY WALL

Since the maggot has no legs to direct its motions, it must accomplish all its actions by movements of its body, but, so well developed to this end are the muscles of its body wall, that no act of shortening or lengthening, no contortionistic twist or turn, is impossible to it.

Plate 4, C, shows the inner walls of the first four body segments of the maggot cut open from above and spread out flat, with the pharynx turned forward, exposing its ventral surface. Plate 4, E, shows the right half and all of the back of two succeeding segments in the same position but somewhat more enlarged. It will be seen that all the muscles of the body wall lie flat against the skin and that most of them form an intricate network of fibers crossing in all directions. Two latero-ventral bands (*VMcl*) are the only longitudinal muscles of the body. In the first three segments these bands are augmented by extra muscles on each side, which become oblique in the fourth segment.

In general, all the longitudinal and oblique muscles are attached at each intersegmental line. In the thorax, however, the longitudinals are continuous from the edge of the first abdominal segment (Pl. 4, C, *I*) to their insertions on the rear margins of the larval head. These muscles are thus made particularly effective as retractors of the head and pharynx. In addition, all of one set of oblique muscles of the second segment are continued through the first to be inserted in a circle around the base of the head and on the roof of the atrium. By the combined action of all of these muscles the head can be entirely withdrawn into the prothorax and the latter itself greatly retracted (Pl. 4, D). The lengthening of the body is accomplished by the constrictive action of the oblique muscles and the resulting pressure of the blood and viscera. The oblique muscles cross one another in two regular systems as shown in Plate 4, C and E, forming a pattern repeated in each segment except the first, where one system is omitted. No muscles, however, cross the back, where the heart lies along the midline (Pl. 4, E, *Ht*) in an open space between the muscles on opposite sides.

Another system of weak, superficial muscles lies close to the skin and consists of a few fibers in each segment disposed more transversely as shown in Plate 4, C and E. Finally, along the sides of the body, there is a row of slender transverse muscles (Pl. 4, E, *TMcl*) placed on the intersegmental lines entad to the attachments of the other muscles attached along these lines.

This complicated muscular sheath of the maggot is a special feature of the larva, since few of its fibers are retained in the adult. Most of the larval muscles are either destroyed by histolysis in the pupa stage or form merely a reconstructive basis for muscles of the imago. Van Rees (*47*) describes only three pairs of muscles in the mesothorax of the larva of the blow fly (Calliphora) as persisting into the adult stage, and Breed (*10*) agrees with him in this, but Pérez (*39*) describes muscles of the abdomen also as going over into adult muscles, though with complete reorganization. Most investigators, however, agree that in the higher Diptera the majority of the muscles of the adult are built up anew from mesoderm cells of the imaginal buds. Whatever may be the truth with regard to details, however, the musculature of the maggot is a striking example of the degree to which organs may be developed for use during one stage of an insect's life and then discarded or reconstructed to serve the needs of the next stage. The histolysis of larval muscles and the histogenesis of the imaginal muscles will be discussed in the section on metamorphosis.

THE CIRCULATORY SYSTEM

The heart and aorta are of ordinary tubular form, and are suspended along the midline of the back between the dorsal oblique muscles (Pl. 4, E, *Ht*) in a delicate diaphragm that lies close to the body wall. The writer has not made a detailed study of the circulatory organs of the apple maggot, since they apparently do not differ from those of other cyclorrhaphus Diptera, and must be studied by means of sections. The dorsal vessel of the fly maggot as described by other writers, Pantel (*37*), Wandolleck (*52*), Lowne (*28*), and Giacomini (*12*), consists of four parts, a posterior section or ventriculus, a median part, the aorta, and an anterior extension of the dorsal wall of the aorta forming an inverted trough-like tongue. The posterior and median sections are contractile and are suspended in the diaphragm, which consists of muscle fibers and the pericardial cells, the latter being larger along the posterior section of the tube than along the median section. Giacomini describes the muscles as ending mesally in branching fibers enclosing the pericardial cells and inserted on the lower lateral parts of the heart.

The most characteristic feature of the circulatory organs of the maggot is the presence of a suspensory ring at the anterior end of the aorta in the neighborhood of the frontal sacs and brain lobes. This was first described by Weismann (*53*) in the blow fly, and has been the subject of many complicated descriptions. Giacomini (*12*) says that in *Eristalis tenax* the ring, or suspensory anellus, is composed of epithelial-like cells and slants from above downward and posteriorly. At each end there is a core of small, compressed cells. The ring is anchored at its lower end by two pairs of tendinous cords. One pair goes back to the dorsal wall of the proventriculus, and the other goes forward to the anterior tracheal commissure and neighboring tracheal branches. The aorta, according to Giacomini, penetrates the ring but its lower lip ends immediately in front of it, while its upper half is continued forward past the upper end of the ring to the anterior dorsal commissure of the tracheal trunk, where it ends in membranous ligaments attached to the brain lobes, to the tracheal trunks, to the frontal sacs, and to the pharyngeal muscles. The posterior end of the heart is suspended from the dorsal wall of the body by delicate tendinous fibers.

THE NERVOUS SYSTEM

The nervous system of the maggot is highly specialized by the condensation of the ventral ganglia. The brain consists of two lobes (Pl. 3, F, *Br*) embracing the œsophagus and connected by a thin bridge above it. There is but one ventral nerve mass, a long, compound ganglion (*Gng*) which is suspended in the body cavity, and extends from the brain to beneath the anterior end of the stomach. This ganglion shows no superficial evidence of its composite origin, except in the numerous paired nerve trunks that arise from it. Only the larger ones of these are shown in the figure.

The unusual position of the brain, in the back part of the thorax (Pl. 3, F, *Br*), is evidently due to the invagination of the primitive head. Since it lies immediately behind the frontal sacs (*FS*) and gives off a short nerve to each optic bud (*ob*), its position is normal in relation to these parts. In the embryo, as shown by Pratt (*41*), the brain lies in the head behind the frontal involutions. The suspension of the ventral ganglion (*Gng*) appears to be for the purpose of allowing its free motion back and forth with the extension and retraction of the pharynx and the forward parts of the body wall.

METAMORPHOSIS

The apple maggot attains its full growth in the fruit where it reaches larval maturity by the end of summer. By this time, however, the decay of the pulp, spreading from the maggot's excavations, has caused the apple in most cases to drop to the ground. The mature maggot tunnels outward in the fruit, makes a small exit hole in the skin, emerges, and enters the earth to a depth of from 1 to 3 inches.

Scarcely, however, has the maggot given itself proper burial when its skin begins to turn yellow, to shrink, and to harden. These changes progress till the creature loses all power of movement and has become transformed, by the end of 12 hours, into a motionless, hard-shelled, seedlike object, brown in color, and only 4 or 5 millimeters long, or about two-thirds the length of the active maggot. The insect in this state is usually called a *puparium* (Pl. 5, A, B), but the term should be used to designate only the hardened larval skin which now forms a tough capsule within which the insect will complete its larval life, go through its pupal stage, and transform to the fly. The puparium is, therefore, not a "stage" of the insect's life history. The short length of the puparium as compared with that of the active maggot is due not only to the shrinking of the cuticle, but also to a final involution of the anterior parts, which results in the complete disappearance of the larval head, and in the infolding of nearly all of the prothoracic segment. As a consequence the anterior larval spiracles (*ASp*) come to project from the anterior lateral angles of the puparium.

The external surface of the puparium retains the larval characters, though some of them in modified forms. The segmentation is still evident and the intersegmental lines are marked by the bands of hooklets, but the swellings have disappeared and the contour is unbroken. Both pairs of larval spiracles are present (Pl. 5, A, *ASp*, *PSp*), but the papillæ about the area of the posterior spiracles are much reduced in size, the smaller ones being scarcely perceptible. The larval mouth is now closed by the hardening of the indrawn parts which form a chitinous plug (*Mth*) in front of the atrium. The anal lobes (*An*) have become densely chitinized and closely appressed, but not fused.

On the other hand certain new characters have developed. During the transformation a ridge swells out on each side of the anterior segments on which there appears a seamlike line (*o*) continuous from one side to the other around the front of the prothorax just below the anterior spiracles. This line ends

posteriorly on each side near the middle of the fourth segment, where it meets a second transverse line (*p*) encircling this segment. These seams are the lines of cleavage along which the puparial shell will be broken open by the escaping fly. Finally, as soon as the puparial cuticle is well chitinized, there appears along each side of the body a row of nine minute stigmata, the primitive lateral spiracles (*Sp*) which have remained rudimentary and invisible during the preceding larval period. The first is located on the methathorax just below the lateral ridge, the next is in the lower angle of the two cleavage lines that meet on the side of the first abdominal segment, and the others follow in line to the eleventh, or eighth abdominal, segment where the last of the series is situated just below the upper of the two lateral papillæ at the sides of the area of the posterior spiracles. These stigmata represent all the adult spiracles except the first, which, though it appears later on the pupa, is apparently obscured by the involution of the prothoracic parts of the puparium.

Though the transformation to the puparium normally goes on beneath the ground, it will take place whether the larva is buried or not so long as it is in an atmosphere sufficiently moist. Hence, for convenience of study, maggots may be collected in a dish as they leave the apples, killed in hot water at desired intervals, and transferred to 85 per cent alcohol, where the puparial shell should be broken. The soft-bodied insect within the puparium can usually be quite easily removed intact, when it is 24 hours old or over, by gently scratching the shell crosswise with a sharp needle till it splits. One end of the capsule can then be taken off and the insect removed from the other.

During the first few hours after the completion of the puparium the hardened cuticle retains its connection with the hypoderm; but at the end of 4 hours in some specimens, later in others, the puparium becomes partly loosened from the inner skin, and after 12 hours it usually forms a free capsule that can be easily removed from the body of the insect within, though it still adheres to the latter at the mouth, anus, and spiracles.

Now, from reading most descriptions of fly metamorphoses, we should expect to find a pupa within the puparium. But the object disclosed in the puparial capsule of the apple maggot at this stage has no character suggesting a pupa. There are no signs of legs or wings; the form is a mere replica of that of the puparium, though smaller in size; and the lateral spiracles are still closed. The new creature, moreover, has a cuticle distinctly its own, characterized, not by any of the former larval characters, but by a uniform, minute papillation all over its surface (Pl. 5, C). Alcoholic specimens show a whitish film between the new cuticle and the loosened puparium, suggesting a coagulated molting fluid. In fact, it is evident that the maggot of the third instar has changed by a puparial molt (*pm*), not to a pupa, but to a *fourth*-instar larva—an intrapuparial, prepupal larval stage (*Ppu*) complete in all respects except for the retention of the stomodeal, proctodeal, and tracheal linings of the third larval instar.

This intrapuparial larval stage of the fly has received scant notice from other writers, and its existence is not generally recognized. Lubbock (*29*), however, in describing the development of Lonchoptera 60 years ago, said: "When the larva is full grown it detaches itself from the skin, which retains its form, and within which the insect changes into a white, opaque grub, consisting apparently of 13 segments, which gradually diminish in size from one end to the other. There are no limb cases. The skin is covered by small papillæ." But De Meijere (*31*) would discredit Lubbock's interpretation of what he saw, and suggests that the "grub" inside the puparial skin was the larva of a parasite. A reinvestigation may prove that Lubbock was correct.

The larva of Rhagoletis remains in this post-puparial instar a varying length of time, but usually not beyond the end of 48 hours from the time it entered the ground. Then begins another molt which ends in the complete shedding of the papillated cuticle and in the disclosure of the true pupa. But the cast-off integument is not broken; it remains as a delicate, transparent pellicle about the pupa in all its phases of transformation. When the adult fly escapes from the puparium it leaves behind, within the puparial capsule, *two* ruptured skins, the cuticle of the prepupal larva and the cuticle of the pupa, the first characterized by its close-set papillation, the second by a fine surface striation.

The membranous pupal envelope has been noted by other investigators. Künkel d'Herculais (*27*) says of the Syrphidae: "The pupa is really enclosed in a very delicate skin, a true chitinous cuticle secreted by the hypoderm at the moment of metamorphosis to ensheath all the members. Within this envelope, so transparent that the development can easily be followed through it, the insect organizes itself into the adult."

The shedding of the prepupal cuticle begins at the anterior end of the body (Pl. 5, D, E) and, as the leg buds and the wing buds are everted, the thorax shrinks, thus creating a considerable space inside the loosened membrane. From its first appearance the pupa suggests the structure of the fly with its large mesonotum (Pl. 5, D, N_2) bordered in front by a narrow pronotum and behind by a rudimentary metanotum. The appendages, however, are small at this early period and there is yet no sign of the future head. By the shrinkage of the thorax in the transformation, the tracheal linings (Pl. 5, E, *tra*) attached to the anterior larval spiracles are partially drawn out of the body of the pupa. The new pupal spiracles (*PuSp*) appear between them on the anterior rim of the pronotum (Pl. 5, D, N_2). The pharyngeal skeleton of the larva, however, remains normally still within the pupa, but since it retains its connection with the puparium, it is always extracted with the latter when the puparial shell is removed, leaving a rupture in the prepupa cuticle. For this reason the atrium (*Atr*) appears in Plate 5, E, as an empty, wide-open hole in the front of the thorax.

The molt progresses backward over the abdomen till finally, at the end of the third day, it forms a complete bag (Pl. 5, F, *ppu*) containing the pupa (*Pu*), but still anchored to the latter by the linings of the tracheae, of the stomodeum, and of the proctodeum, and attached to the puparium (*pm*) at the spiracles, mouth, and anus. The abdomen has the prepupal form, but there are no posterior spiracles replacing those of the larva as on the prothorax. There is still no head visible. This phase of the pupal development is the one described by Wahl (*51*) as the *cryptocephalic* stage.

The pupa remains in this substage (Pl. 5, F) for a variable period of time, and at first can be easily removed from the puparium in the headless form. But, a little later, the slightest pressure consequent on handling causes the head to pop out of the hole in the anterior end of the thorax, when the pupa suddenly assumes the grotesque aspect shown in Plate 6, A. The head is so easily forced out at this period that it can not be demonstrated that its eversion at this time is a normal phase of development, but the appearance of the head marks the beginning of the second substage of the pupa, the one called by Wahl (*51*) the *phanerocephalic* stage, which in its final phase is shown in Plate 6, C and D.

When the head is first exposed it is very small (Pl. 6, A) as compared with its eventual size (Pl. 6, D). It is a tense, thin-walled capsule with the ptilinum everted in the form of a caplike lobe (*Pt*) on the anterior dorsal aspect. Just below the ptilinum is a transverse, bilobed ridge (*Ant*) within which are developed the antennæ of the fly. On the sides of the head are the compound eyes (*E*), very small at this period, and below each of them a swelling (*u*) which is more developed in the next phase (Pl. 6 C, D), but which is lost in the adult. The

legs and wings (Pl. 6, A) are longer than in the cryptocephalic period (Pl. 5, F), and the thorax shows more structural details of the adult. The abdomen is still very large and completely fills the posterior two-thirds of the puparial capsule.

The walls of the head have been derived from all the parts that were formerly invaginated in the embryo and larva, including the frontal sacs, the atrium, and the dorsal pouch of the latter. The eversion of the head pushes out the skeleton of the larval pharynx, and the entire lining of the pharynx and oesophagus comes out with it; but the larval linings of the tracheae and the intestine still remain within the body of the pupa. When the puparium is artifically removed, however, the intestinal lining usually comes out attached to it, though the dorsal tracheal tubes (*tra*) frequently break loose and remain only partly extracted.

This small-headed phase of the pupa is but a transitory period and is rapidly passed over. In fact, the writer never found intervening phases between it and the next when the head has acquired its final external form and size (Pl. 6, C,D). This arouses suspicion that the evagination of the head at this time may be in all cases a result of the handling of the specimen. Yet the fully formed head is so large in proportion to the size of the thorax that it does not seem as if it could have developed to this degree without a natural eversion at an earlier period of growth.

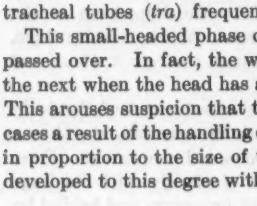

FIG. 7.—Head of an adult fly with subocular horns, *Elaphromyia cervicornis* Saunders. (From Saunders, *43*.)

In the final phase of the phanerocephalic pupa (Pl. 6, C, D) the head constitutes almost a third of the entire bulk of the insect. The abdomen has contracted much in size, but still shows the 8 segments of the larva and, in addition, a small anal segment differentiated at the end. The thorax, wings, and legs have greatly increased and lengthened. The ptilinum (*Pt*) is now relatively small, but the bilobed, antennal ridge (*Ant*), the compound eyes (*E*), and the subocular lobes (*u*) are much larger. Each of these last consists of a thick basal part and a tapering terminal spur directed backward. Künckel d'Herculais (*27*) noted similar subocular processes in the Syrphidae, which he says are peculiar to the pupal stage in this family, though in several tropical species of Terastiomyia and Elaphromyia they are present in the adult and developed into long appendages. One such species, *T. lobifrons* Bigot, is described and figured by Bigot (*6*) as having long, bent chitinous processes extending downward from the cheeks. Other forms are described by Saunders (*43*). One of these, *E. cervicornis* Saunders (fig. 7), has long forked horns like the antlers of a deer; another, *E. alcicornis* Saunders, has large platelike subocular appendages suggesting the horns of a moose. According to Dr. J. M. Aldrich those genera probably belong to the family Micropezidae.

All pupæ of the apple maggot were found to be in the final phase of the phanerocephalic substage at the end of five days, though some mature earlier. Specimens killed at the end of three and four days were found in all of the last three phases of development, the cryptocephalic (Pl. 5, F), the microcephalic (Pl. 6, A), and the macrocephalic (Pl. 6, C). Some of them transformed to flies within a few weeks, while others did not, but this variation is normal, and none was found to remain in a larval stage through the winter, as one writer has claimed.

At some time between the first appearance of the larval head and the attainment of the final pupal form, the larval linings of the tracheæ and usually the lining of the intestine are at last cast out of the body of the pupa. From now on the prepupal cuticle (Pl. 6, G, *ppu*) always shows the molted tracheal linings stuck flat against its inner surface. Those from the anterior spiracles (*ASp*) extend backward as two conspicuous tubes lying just below the level of the

horizontal cleavage lines of the puparium; those from the nine lateral spiracles (*Sp*) form a tangled mass of threads along each side; while those from the hind spiracles (*PSp*) are disposed against the dorsal wall of the envelope in the form of a double-stalked T, as shown in Plate 6, E (*tra*). The extraction of the anterior and the lateral tubes may be accounted for by the contraction of the pupa, but it is difficult to understand how the posterior tubes can acquire their curious arrangement (Pl. 6, E) unless the pupa sways its abdomen sidewise to extract them.

The pupa, now entirely free from its envelopes, retains no connection whatever with any of the larval spiracles, and its own spiracles (Pl. 6, D, *PuSp*) have no direct access to the outside air. In the Anthomyiidæ the hornlike pupal spiracles are said to penetrate the puparium between the first and second abdominal segments. In *Rhagoletis*, however, as the body of the pupa contracts during its transformation, the space about it, within the prepupal skin, becomes filled with gas which is visible as a large bubble through the walls of the puparium, and which gives the insect at this stage such buoyancy that it will float on the surface of water. This gas is very evidently the respiratory medium of the pupa, though it is puzzling at first to account for the presence of air in the pupal chamber. An examination of the molted anterior tracheal tubes, however, will suggest the mode of its ingress. These tubes (Pl. 6, F, *tra*), always keep a firm hold on the stigmatic chambers of the spiracles (*ASp*), and the walls of their anterior parts are so rigid that they remain as open cylinders filled with air as far back as the rear margin of the second segment. In the third segment, however, the tubes abruptly collapse to flat bands of flabby tissue into which no air penetrates; but, just before this point, there is always a rupture (*w*) on the inner face of each tube, and these holes establish free passageways from the anterior larval spiracles (*ASp*) of the puparium (*pm*) into the pupal chamber within the prepupal envelope (*ppu*), evidently the inlet of the pupa's supply of air. The slowly diminishing size of the pupa during the period of its transformation may be assumed to suck in the air that forms the first bubble, but subsequent renewal must be by diffusion through the spiracles.

The pupal spiracles differ from the corresponding larval prothoracic spiracles in being bilobed (Pl. 2, A). The stigmatic chamber of each is elongate and forks into the two spiracular lobes where it ends in numerous small papillæ that press against the cuticle and appear to contain perforations through the latter. Each pupal spiracle is developed mesad of the preceding larval spiracle (Pl. 5, E), and the remains of the larval stigmatic chamber form outer and inner scars (Pl. 2, A, *m*, *n*) with a cuticular strand (*v*) connecting them as in the larval instars.

After the fifth day the pupa undergoes no further external change. Some pupæ will transform to adults in the fall of the same year, but the majority remain in the pupal stage till the following year, when the flies begin to emerge about the first of June, while a few, it is said, do not transform till the second year after the end of the larval stage.

The external forms of the pupa show the progress of the change from the larva to the imago; the real activities of reconstruction go on beneath the surface. The remaking of the insect is not accomplished in the manner by which a piece of clay may be remodelled from one form to another. The processes of metamorphosis are physiological and consist of an active metabolism that affects nearly all the tissues, but not all in the same way or to the same degree. Some parts simply awake from a dormant state and pursue their normal course of development by a sudden access of growth; others that were functional in the larva are altered by direct growth resulting from the activity of their own cells. But the changes most characteristic of metamorphosis are those involving coordinated histolyses and histogeneses resulting in the destruction of larval

tissues and their replacement by new ones generated from the imaginal buds. Finally, some organs undergo histolysis alone and are not replaced, while others are newly formed in the pupa by simple histogenesis. These various processes, however, as we shall see, are probably not so diverse as they have seemed.

The best examples of parts that follow the path of normal development from embryo to imago are the organs of reproduction. But even here growth is almost arrested during the larval period. In the pupa the organs resume activity, but in the case of the apple maggot, according to Illingworth (*15*), the ovaries of the female fly do not produce mature eggs till from 14 to 24 days after emergence.

The heart, the nervous system, and the tracheal system undergo more or less change in the pupa in order to arrive at the forms they are to have in the fly. Yet, in most cases, the metamorphosis of these organs is a matter of direct transformation by the growth of their own tissues. Alterations of this sort take place also in insects with incomplete metamorphosis, and constitute a bond of unity between the young of such insects and the maggot, grub, or caterpillar of insects with metamorphosis erroneously called "complete."

The larval hypoderm, salivary glands, and alimentary canal have their tissues destroyed but replaced in situ by new ones proliferated from the imaginal buds, and such regenerated parts often take on forms strikingly different from those of the larva. Many of the muscles undergo a similar metamorphosis, though the new tissues have never been traced to imaginal muscle buds, being formed by a reorganization of the elements of the old muscles. This process, however, is apparently not confined to the pupal stage, for Mobusz (*35*) has described the regeneration of the stomach epithelium in the larval instars of a beetle (Anthrenus) as well as in the pupa. It may be suspected, therefore, that reconstruction may occur also in insects with incomplete metamorphosis.

Many of the larval muscles, especially in the higher Diptera, are completely destroyed by histolysis and are not replaced by corresponding new ones. On the other hand, some muscles that have no larval prototypes are newly generated in the pupa. Those of this type most probably arise from the mesoderm layer of the imaginal discs of the body wall. The destruction and regeneration of muscles must take place also in some insects with incomplete metamorphosis, but the histology of the process has not been studied in this group.

The metamorphosis of the hypoderm and of the alimentary canal has been described by so many writers, who agree on most of the details, that only a general statement need be made here. The writer has not studied the subject in Rhagoletis. At the beginning of metamorphosis the ectodermal cells of the imaginal buds of the hypoderm begin to divide and multiply. Wherever there are appendages the latter grow rapidly, but the peripheral parts of the buds spread out and constitute the new hypoderm of the body wall. The old cells and the basement membrane are absorbed into the blood or are devoured by phagocytes as the new tissues crowd upon them and take their places. This is subsequent to the loosening of the larval cuticle, though the latter may not be cast off till some time later.

Likewise, the epithelium of the foreintestine and of the hind intestine is replaced in the same way from imaginal buds in the intestinal walls. In the midintestine, or ventriculus, however, the larval epithelial cells are shed into the lumen, and the imaginal epithelium is regenerated from groups of deep-seated regenerative cells. According to Rengel (*42*) these regenerative cells in a beetle (*Tenebrio molitor* L.) are the same that also regenerate the ventricular cells lost during the process of digestion in both the larval and the adult periods. The new epithelium proceeds at once to digest and absorb most of the discarded larval epithelium.

All of these tissues are regenerated without destroying the continuity of the organs concerned. The pupa is never without a hypoderm, never without an alimentary canal (Pl. 6, B). The new cells advancing from the imaginal buds follow closely on those disappearing, or overlap them. The new forms of the organs result from the forces of heredity inherent in the new cells or somehow imposed upon them.

In the organs of mesodermal origin, however, especially in the muscles, the tissues are much more profoundly affected by the processes of metamorphosis; yet, even here, in the simpler forms of metamorphosis, the muscles are mostly remodelled *in situ*, and in the higher flies, where the general dissolution is most intense, some of the muscles are said to be modified only by the addition of new fibers. Most of the larval muscles in the Muscoidea, however, go into complete histolysis, while the new ones are built up with little relation to those of the larva.

In the apple maggot the beginning of histolysis in a muscle is first evident in a few fibers that lose their cross striation and appear to melt together into a homogeneous granular mass. This mass then breaks up into small fragments, which are at first densely packed but which are soon dissociated by a clear substance that appears among them as if formed from the liquefying of some other parts of the muscle tissue, probably the sarcoplasm. In this way the muscle disintegrates, resulting in the appearance of clear plasmic areas along its edges and between its fibers, filled with granular fragments of the formerly striated tissue. As dissolution progresses deeper and deeper into the body of the muscle, the plasmic lacunæ enlarge and engulf an ever-increasing number of the fragments. Finally, the lacunar plasma dissolves along its outer edges and the muscle fragments float off free into the blood.

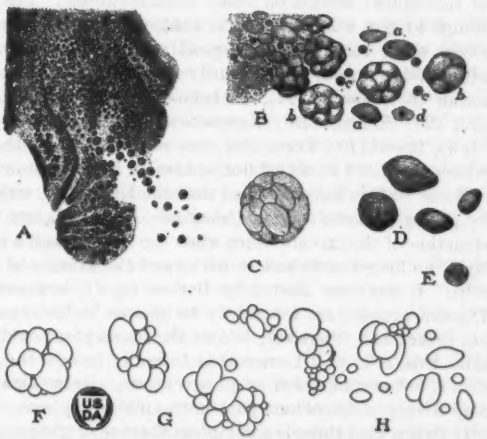

FIG. 8.—Histolysis of muscle tissue. A, piece of pharyngeal muscle in histolysis just after formation of puparium, showing disintegration of muscle into fragments or *sarcolytes* and the liberation of the latter at the dissolving edge. B, showing various elements given off from muscle in histolysis; *a*, free muscle fragments or sarcolytes; *b*, globules of fragments; *c*, free muscle nuclei; *d*, a muscle "cell" or muscle nucleus in a piece of muscle substance. C, a globule of sarcolytes, about 34 microns in diameter. D, free sarcolytes or muscle fragments, 25 to 30 microns in length. E, cell containing several small bodies, perhaps a free muscle nucleus after division into secondary nuclei, 8 to 10 microns in diameter. F, sarcolyte globule in early stage of disintegration. G, globules in more advanced stages of disintegration. H, globules in last stages of breaking up into free sarcolytes

Among the bodies in the plasmic areas of a disintegrating muscle we may distinguish several sorts. The most numerous are simple, oval, granular pieces of the fiber substance, the *sarcolytes* of other writers. Then there are smaller bodies containing nuclei, virtually nucleated cells derived from the muscle tissue. These are the *caryolytes* of Berlese (*4*, *5*). Finally, there are often large numbers of free muscle nuclei, or nuclei surrounded by only a very small amount of clear protoplasm. Figure 8, A, shows a piece of a larval pharyngeal muscle in which histolysis is well advanced, and in which is seen the formation of the various elements of disintegration, first deep in the muscle tissue, then liberated into the

clear plasma of the lacunæ, and finally given off at the dissolving edge of the latter into the blood.

The different kinds of bodies given off from the edge of a dissolving muscle are shown enlarged at B in figure 8. Here are seen free sarcolytes (*a*), a caryolyte (*d*), and free muscle nuclei (*c*, *c*). It is to be noted, however, that the majority of the sarcolytes are given off in small globular masses (*b*, *b*) which are extruded from the muscle, apparently in a thin matrix of plasma. These bodies soon become spherical (C) and are very suggestive of the *Körnchenkugeln* or *spherules of granules* of other writers.

A "Körnchenkugel" is supposed to be a leucocyte or amoeboid blood corpuscle gorged with the fragments of disintegrating larval muscles, which is to say, it is a phagocyte after a full meal. The term was first used in this sense by Weismann (*53*), but the idea has given rise to an endless discussion in the works of subsequent writers on insect metamorphosis. The present writer will not attempt to give a history of this subject here, since the reader will find in almost every other recent paper a good review of the results and conclusions of all other writers, and, if sufficiently interested, should consult the works of Weismann (*53*), Ganin (*11*), Metschnikoff (*32*), Van Rees (*46*, *47*), Kowalevsky (*24*, *25*), Rengel (*42*), Karawaiew (*17*), Needham (*36*), Anglas (*1*), Berlese (*4*, *5*), Breed (*10*), Pérez (*39*), and other papers by these same and other writers which are listed in all bibliographies on insect metamorphosis.

Some writers have believed that the leucocytes, either acting as phagocytes or by giving off some solvent (*lyocytosis*, Anglas, *1*), are the actual cause of the destruction of the larval tissues when the latter reach a condition where their vitality is no longer sufficient to withstand the attacks of these ravenous or virulent cells. It has been shown by Berlese (*4*, *5*), however, that even in the higher Diptera muscles are frequently to be seen in histolysis when the sarcolemma is yet intact and, therefore, before the leucocytes could attack their tissues. Besides this, it is well known that in many insects the metamorphosis of the tissues is not accompanied by phagocytosis. Karawaiew (*17*) says that phagocytes play a very unimportant part in the metamorphosis of the ant, while Korotneff (*21*) claims that there is a complete absence of phagocytosis in Tinea, and Rengel (*42*), Needham (*36*), and Breed (*10*) report the same for various beetles.

Even in the Diptera the presence of phagocytes is not universal. Miall and Hammond (*34*) say that they "have never seen in Chironomus larval muscles excavated by phagocytes, nor fragments of striped muscles lying inside phagocytes, though both can be demonstrated in the blow fly. In Chironomus the disintegration of the larval organs of the thorax is relatively slow, and the muscles, for instance, seem to waste gradually and uniformly while undergoing for a long time no marked change of external form." Kellogg (*18*) has shown also that in Holorusia and in Blepharocera histolysis takes place without the occurrence of phagocytosis, and Berlese (*5*) says the same of Mycetophila. Yet Pérez (*39*) still insists that, in the blow fly, phagocytosis is at least synchronous with histolysis, and he inclines to the belief that the attack of the leucocytes is the actual cause of the destruction of the muscles. Probably most investigators, however, now regard the leucocytes as mere scavengers that devour the products of histolysis, the latter resulting from some other unknown cause. Most writers believe that the phagocytes digest the engulfed particles and give the products back to the blood in a form that can be utilized by the growing tissues. Berlese, on the other hand, claims that they act as carriers only.

Nearly all writers have admitted that, where the leucocytes play the part of phagocytes in the higher Diptera, they form the bodies known as "Körnchenkugeln" or "spherules of granules;" but Berlese has shown that similar bodies

may be formed from aggregations of sarcolytes, though he claims that such bodies contain at least one nucleated fragment. Janet (*16*), however, says that in the Muscidæ some of the spherules are mere masses of sarcolytes which may or may not contain a muscle nucleus.

In the pupa of Rhagoletis there are great numbers of spherules of muscle fragments (fig. 8, C) of the sort described by Janet, and the writer did not certainly find any containing nuclei. Moreover, many of these spherules are formed deep in the body of the disintegrating muscle (A) where no leucocyte could get at them. They originate as mere groups of sarcolytes that adhere in small masses as the sarcoplasm dissolves about them, and that have been thus set free with a thin plasmic matrix cementing their elements together in globular form. Furthermore, leucocytes are always scarce; never are they found in such numbers as to account for the great mass of spherules that is eventually given off into the blood.

The sarcolytes have been described by nearly all writers as retaining the original striation of the muscle fibers. In Rhagoletis the muscles lose their striation at the beginning of histolysis, and the sarcolytes are at first granular, though they soon acquire a striated appearance (fig. 8, C, D). Their "striation," however, is clearly secondary and appears to be due to a puckering of the surface, since the lines are always lengthwise and converge at the poles of the oval fragments. The material studied was taken from muscles or from drops of the body liquid of freshly killed larvæ. The specimens were not fixed or hardened, and some were examined without staining, in order to be sure that nothing could be attributed to the effects of reagents.

The bodies derived from the disintegrating muscles soon become very abundant in the blood. The spherules of sarcolytes are the most numerous and the most conspicuous, and most of them remain intact for a long time. After the pupa has acquired its final external form, however, they begin to disintegrate in a manner similar to that by which the fat cells go to pieces. They first lose their even contours (fig. 8, F), and later appear in very irregular forms or as mere loose aggregations of sarcolytes (G). This condition results evidently from a dissolving of the plasmic matrix that so far has held the component fragments together. By the fourteenth day after the formation of the puparium most of the sarcolyte spherules are in these various stages of disintegration.

At about this time the blood of the pupa has a thick, granular, creamy consistency, so charged is it with the débris of both the muscles and the fat cells. Among this material there may be distinguished fat cells still intact, fat cells in various stages of disintegration, free oil droplets, masses of proteid granules from the fat cells, sarcolyte spherules still intact, spherules in all stages of dissolution, free sarcolytes, caryolytes, and free muscle nuclei. In addition, there are leucocytes, various unidentified cellular elements, and great numbers of small grains of indeterminable origin.

At the end of 18 days the sarcolyte spherules have been mostly broken up, and only free sarcolytes or dissolving groups of them (H) remain. The fat cells have likewise in large part disappeared. If the pupa is to become a fly in the fall of the same year, the contents of the blood are now rapidly dissolved; but with those that will not transform till the following summer, the processes of absorption are slower, and by the first of December the blood is still thick with the residue of larval tissues.

Frequently there are to be seen in the blood small cells of the size of muscle nuclei, which contain several smaller bodies (E). These are evidently what Berlese (*5*) calls *sarcocytes*, or larval muscle nuclei that have divided by direct division into a group of small nuclei. These secondary nuclei, he says, become

liberated and grow into elongate migratory cells, the *myocytes*, which gather at the points where new muscles are to be, or group themselves with the remaining parts of old muscles, to build up the muscular system of the imago. Anglas (*1*) described the imaginal muscles of the wasp as originating from *myoblasts*, or fragments of the larval muscle nuclei in pieces of the contractile tissue.

Ganin (*11*) and Van Rees (*47*), however, had described the imaginal muscles of the blow-fly as formed in part or entirely from cells proliferated from the mesodermic tissue of the imaginal buds of the body wall. Pérez, in a more recent study of the metamorphosis of the blow-fly (*39*), reasserts this same view concerning the origin of the new muscles or of new parts of old ones, and claims that the larval muscle nuclei finally perish by phagocytosis.

One of the most convincing papers on the metamorphosis of the muscles is that by Breed (*10*) in which are described the changes that occur in the muscles of certain beetles. In the species studied, Breed found no evidence of phagocytosis, and, according to his observations, muscles that metamorphose are reconstructed from their own elements, not from migratory "myocytes," while muscles of new formation are probably developed from the mesoderm of the imaginal buds. Cells of external origin do penetrate the growing muscles, but these, he points out, are derived from neighboring tracheole cells and form the imaginal tracheal branches of the muscles. Such cells, Breed suggests, are the ones observed by Berlese and described as migrating "myocytes" on the mistaken belief that they formed the new elements of the muscles. Breed's account agrees entirely with that of Rengel (*42*) on the metamorphosis of the muscles of the alimentary canal. Needham (*36*) says that some of the nuclei of the degenerating fat cells of the flag weevil become associated with the developing muscles, apparently themselves becoming nuclei of the new muscle fibers. This, however, seems no more reasonable than Berlese's idea, later discarded by himself, that some of the muscle nuclei form the imaginal fat cells.

The muscles of any pupa, then, may be classed in not more than four groups, as given by Breed (*10*). The first includes such muscles as pass unaltered from larva to imago, but most writers agree that there are no muscles of this sort in the higher Diptera. The second group includes such larval muscles as undergo a more or less complete metamorphosis by a reorganization of their own elements. Van Rees (*47*) described three pairs of larval muscles in the thorax of the blow-fly that persist with alterations, and this is confirmed by Breed. The third group consists of those larval muscles that disappear by complete histolysis. This includes most of the larval muscles of the Muscoidea. The fourth group consists of muscles that are newly formed in the pupa from preexisting imaginal buds, and includes most of the muscles of the adult fly.

The muscles of new formation are to be regarded as present in rudimentary form in the imaginal buds. These muscles are those that the larva has found unnecessary or unsuited to its purposes, and, as stated by Breed, "such muscles would tend naturally to be retarded in their development until they came to be muscles newly formed in the pupa; but in their final development they would arise from the cells which had originally formed them." It has not been shown yet just what elements in a metamorphosing muscle generate the new parts, but the evidence is against the idea that regenerating elements enter from without, and Breed sees no evidence that the new elements in such cases are derived from the imaginal discs. Perhaps each muscle, then, contains cells with persistent vitality that regenerate it, just as the hypoderm and the walls of the alimentary canal are regenerated by cells in their own tissues that have remained dormant through the larval stage or that have retained their vitality into the pupal period.

SUMMARY

By assembling all available information on the comparative anatomy, embryology, and postembryonic development in the Diptera, we may construct the following outline of the evolution of one of the higher flies, and thus arrive at a better understanding of the structure and metamorphosis of a maggot than could be gained from a study of the maggot alone.

1. The larvæ of primitive flies had legs and a normal head with antennæ and biting mouth parts, and breathed through lateral spiracles connected with the lateral tracheal trunks.

2. As a result of the reduction of the external appendages in the larval stage, the corresponding appendages of the pupa were forced to back into the body in order to find space for growth, thus coming to be developed in deep pockets of the hypoderm.

3. As long as there was a remnant of the larval appendage, the tip of the pupal appendage developed within it. When the larval appendage disappeared entirely the bud of the pupal appendage became free in its hypodermal pouch. The bud was then at liberty to begin its growth in earlier larval instars or even in the embryo. Thus has been established the precocious origin of imaginal buds in the Diptera. Yet the imaginal rudiment, regardless of when it begins its growth, is morphologically of the pupal stage—the hypoderm pouches of the buds, the peripodal sacs, shed no cuticle, so far as known, till the molt of the pupa.

4. Imaginal buds of the body wall that do not form appendages, such as most of those of the abdomen, remain as flat discs of cells in the hypoderm, typically a dorsal and a ventral pair in each segment in line with the appendage-forming buds of the thorax.

5. In the higher Diptera the imaginal buds of the antennæ and the compound eyes are developed in a single pouch on each side of the head, the so-called frontal sacs.

6. By an invagination of the frontal region the roots of the frontal sacs were carried inward. These new cavities then united basally, forming a median bilobed pouch with the true frontal sacs arising from the inner ends of its wings.

7. The head of the larva suffered a reduction after the loss of its appendages, and then suffered an involution resulting either from a retraction of the mouth or from a forward growth of a fold from the base of the head. The inturned parts thus came to form an anterior addition to the pharynx, known as the head atrium; and the median frontal pouch, carried in at the same time, formed the dorsal pouch of the atrium. The two wings of the pouch still carry the bases of the original frontal sacs, which last are buried deep in the thorax along with the brain lobes retracted in advance of them.

8. By the involution of the head, the labrum and the buds of the mouth parts were also carried inward. The labrum now forms the roof of the atrium behind the root of the dorsal pouch, while its muscles constitute the dilators of the new larval pharynx. The buds of the mouth appendages arise from the floor of the atrium.

9. The maggot early lost its true mandibles, but later acquired substitutes in the form of hooks located at the sides of the new mouth. Since this region came from the back of the head, or from the neck, the oral hooks appear to be secondary cuticular organs. They move in a vertical plane and are solid structures shed with each larval molt.

10. In connection with the oral hooks there was developed a chitinous skeleton in the walls of the atrium, the dorsal pouch, and the original larval pharynx, thus unifying all these parts in a structure designed to support the hooks and to give attachment to muscles for moving them. This skeleton is also renewed with each larval molt.

11. The original maggots perhaps took to living in soft mud or in water, and, in adaptation to their new environment, they developed a pair of dorsal spiracles at one or both ends of the body in connection with the *dorsal* longitudinal tracheal trunks, while the primitive spiracles of the *lower* or *lateral* tracheal trunks were closed. They thus became propneustic, metapneustic, or amphipneustic by a *new* breathing system, *not by a modification of the primitive peripneustic system.* The dorsal spiracles are independently renewed with each molt.

12. The puparium of the higher Diptera is the hardened skin of the last active larval instar. In Rhagoletis it is loosened from the underlying hypoderm, and a fourth larval instar is formed within the puparial shell. This larva has a minutely papillated cuticle of its own, but it does not shed the linings of the tracheæ and alimentary canal of the third instar. This prepupal larva soon molts in its turn, whereupon the true pupa is formed, and its unbroken cuticle remains as a membranous envelope about the pupa within the puparial capsule. How far this condition is general in the Diptera has not been determined.

13. In the pupal stage all the sunken imaginal parts are everted, including the frontal sacs and the buds of the mouth parts, legs, and wings. Structures that have been acquired by the larva for its own special purposes are lost by histolysis; those that have been modified are remodeled into imaginal organs; and imaginal organs or tissues that have been suppressed grow rapidly into the adult form. In the higher Diptera the pupa goes through two external phases in its development, a cryptocephalic substage and a phanerocephalic substage.

14. The pupa supplants the anterior dorsal larval spiracles with a corresponding pair of its own, but it loses, without replacing, the posterior larval spiracles. In Rhagoletis the pupa obtains air from the anterior larval spiracles of the puparium conducted into the pupal chamber through the cast larval tracheal tubes which remain connected with the anterior larval spiracles and which are ruptured within the prepupal envelope of the pupa.

15. The pupal metamorphosis is for the purpose of restoring the ancestral form of the insect, but it involves a restoration complicated by the addition of all the special characters added by the adult during its own evolution. In general, the more the larva has departed from the ancestral path, the more the adult has modified its inheritance, and consequently the more arduous becomes the work of the pupa in making the one over into the other. Nowhere in insects has this independence between young and adult been carried to such a degree as in the higher Diptera, and consequently a more intense reconstruction goes on in the fly pupa than in any other.

16. Physiologically the life of the insect is divided into but two periods—the larval period and the imaginal period. The pupa is a constructive proimaginal stage separated from the mature imaginal stage by a secondarily acquired molt. It is not a preimaginal phylogenetic stage. In most cases it has developed special characters of its own, and often retains one or more special characters of the larva.

17. The adult represents the primitive form of the insect on which has been built up the special adult characters acquired by each species in its evolution. In insects with metamorphosis the adult restores characters of its ancestors that were discarded by the larva. In the higher Diptera, for example, the fly abolishes the breathing system established by the larva, reduces the enlarged dorsal tracheal trunks to normal size, and reopens the primitive lateral spiracles along the lower lateral tracheal trunks. These spiracles remain through the larval stage as mere groups of special cells in the hypoderm, which appear first, in Rhagoletis, as minute stigmatic spots on the puparium. The imago of insects with complete metamorphosis is not a new creature built up independently from embryonic cells; its first stage, the pupa, follows the last larval stage by a process of replacement which is only a modification of that which takes place between any two larval stages, and which again occurs in normal form at the molt between the pupa and the adult.

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PLATE 1

Rhagoletis pomonella

A.—Lateral view of mature larva in third instar. × 10.

B.—Ventral view of larval head, showing mouth, oral hooks, and sense organs.

C.—Sense organs of snout of larva, greatly enlarged.

D.—Vertical view of same sense organs as in C.

E.—Sense organ at angle of mouth with pair of chitinous teeth on adjacent edge of lip. (See B.)

F.—Part of an intersegmental band of hooklets on cuticle of larva.

G.—Two hooklets seen in profile.

H.—Anterior spiracle of second-instar larva, lateral view.

I.—Diagrammatic dorsal view of anterior spiracle of third-instar larva, showing closed passage from base of stigmatic chamber to cuticle of body wall.

J.—Lateral view of anterior spiracle of third-instar larva.

K.—Three stigmatic papillæ of anterior spiracle of third-instar larva, showing terminal slit in each.

L.—Oral hook of first-instar larva.

M.—Oral hook of second-instar larva.

N.—Oral hook of third-instar larva.

EXPLANATION OF SYMBOLS

1, prothorax.

2, mesothorax.

3, metathorax.

I, first abdominal segment.

VIII, eighth abdominal segment.

An, anus.

ASp, anterior larval spiracle.

BW, body wall.

g, anterior sense papilla on snout of head.

h, posterior sense papilla on snout of head.

Hk, oral hook.

i, sense organ at side of mouth.

j, teeth on edge of lateral hook pocket of mouth.

k, median ridge on roof of larval mouth.

l, median ridge on floor of larval mouth.

LH, larval head, external remnant of invaginated true head.

m, outer stigmatic scar, or closed outer end of passage through which tracheal cuticle of preceding instar was shed with cast-off spiracle.

Mth, larval mouth.

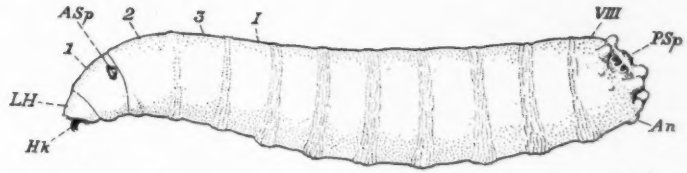
n, inner stigmatic scar, in base of stigmatic chamber. (See *m*.)

PSp, posterior larval spiracle.

SpC, stigmatic chamber.

Tra, dorsal longitudinal tracheal trunk.

v, remains of passage of stigmatic chamber to body wall, through which tracheal intima of last larval instar was shed. (See *m* and *n*.)



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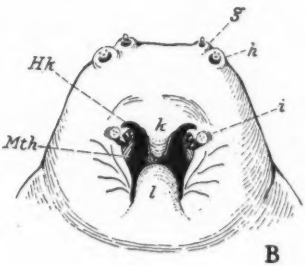

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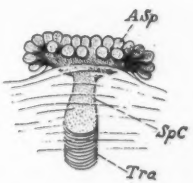

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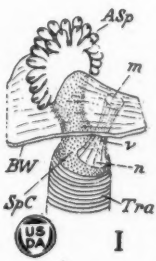

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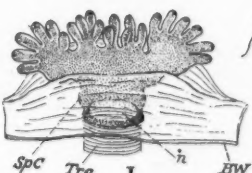

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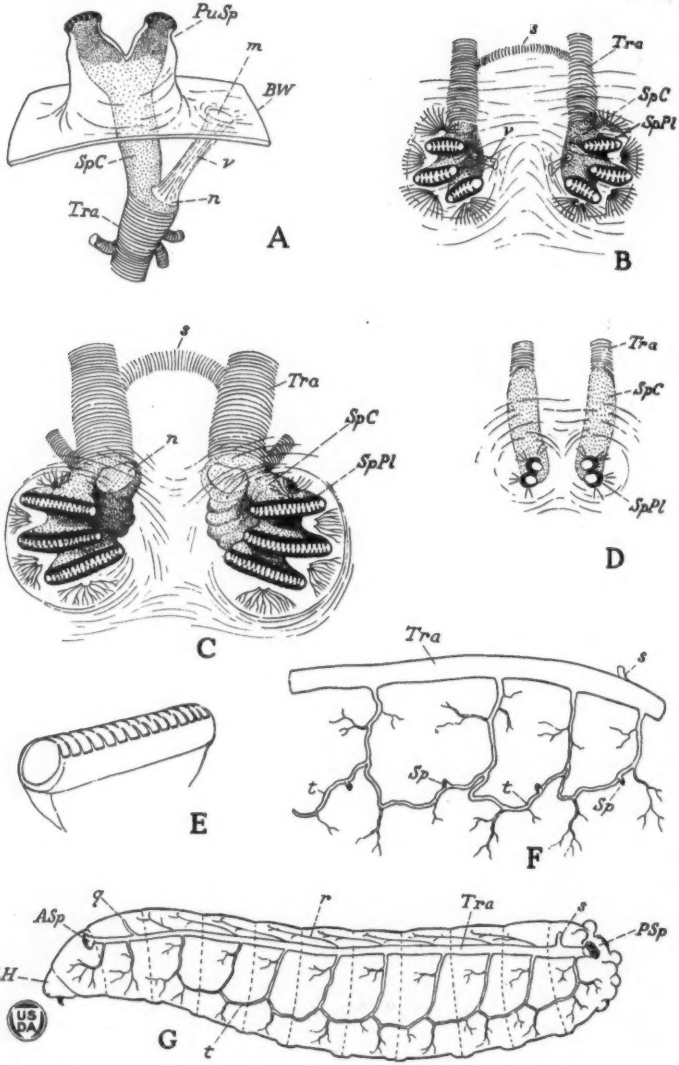
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PLATE 2

Rhagoletis pomonella

A.—Dorsal view of right pronotal spiracle of pupa, showing passageway from base of stigmatic chamber to cuticle through which larval tracheal intima was shed attached to larval spiracle.

B.—Posterior spiracles of second-instar larva.

C.—Posterior spiracles of third-instar larva.

D.—Posterior spiracles of first-instar larva.

E.—Stigmatic plate at end of one lobe of stigmatic chamber of third-instar larva.

F.—Dorsal tracheal trunk and lateral branches of last four segments of prepupal larva, showing lateral spiracles connected with lateral commissures, which will constitute the lower lateral longitudinal trunk of the adult tracheal system.

G.—Principal trunks and branches of left half of larval tracheal system, third instar.

EXPLANATION OF SYMBOLS

ASp, anterior larval spiracle.

LH, larval head, external remnant of the invaginated true head.

m, outer stigmatic scar, or closed outer end of passage through which tracheal cuticle of preceding stage was shed with cast-off spiracle.

n, inner stigmatic scar, in base of stigmatic chamber. (See *m*.)

PSp, posterior larval spiracle.

PuSp, pupal dorsal spiracle of pronotum.

q, anterior dorsal tracheal commissure.

r, V-shaped dorsal tracheal commissures. (Only left halves shown.)

s, posterior dorsal tracheal commissure.

sp, *sp*, lateral spiracles, the adult system, connected with *lower* longitudinal tracheal trunks.

SpC, stigmatic chamber.

SpPl, stigmatic plate.

t, *t*, tracheal commissures forming lower lateral longitudinal trunks of adult.

Tra, dorsal longitudinal tracheal trunk.

v, remains of passage from stigmatic chamber to body wall, through which tracheal intima of last stage was shed. (See *m* and *n*.)

PLATE 3

Rhagoletis pomonella

A.—Pharyngeal skeleton and associated parts, lateral view, mature larva of third instar.

B.—Larval pharynx and left oral hook. Same as A with soft parts removed.

C.—Larval pharynx and skeleton with oral hooks, diagrammatic.

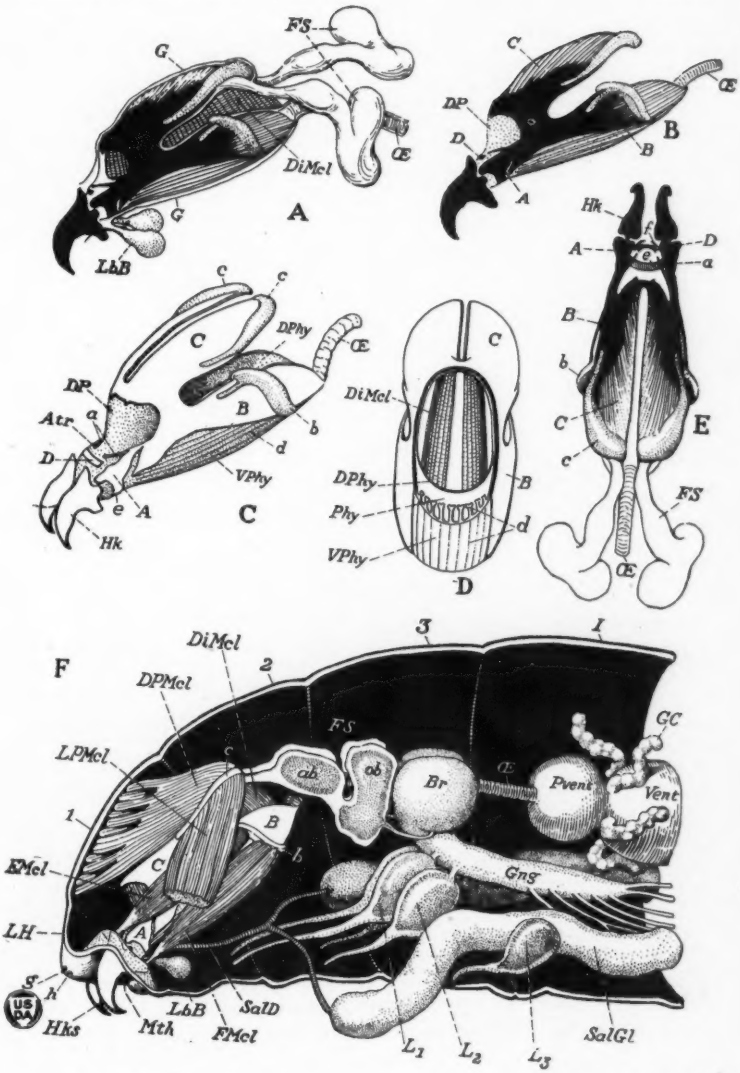
D.—Transverse section of larval pharynx and pharyngeal skeleton through bases of wing plates, anterior view. (Compare with C.)

E.—Dorsal view of pharyngeal skeleton, with oral hooks and frontal sacs, third-instar larva.

F.—Vertical, lengthwise section of head and first four segments of mature maggot, showing pharyngeal skeleton and musculature, the frontal sacs against the brain lobes, imaginal buds of labium and legs, ventral ganglion, salivary glands, and anterior parts of alimentary canal (*Œ*, *Pvent*, *Vent*).

EXPLANATION OF SYMBOLS

1, prothorax.
2, mesothorax.
3, metathorax.
I, first abdominal segment.
A, anterior lateral or triangular plate of larval pharyngeal skeleton.
a, arms of plate *A* in roof of atrium.
ab, antennal bud in frontal sac.
Atr, atrium, anterior, part of larval pharynx resulting from involution of original head of larva.
B, posterior lateral plate of pharyngeal skeleton.
b, ridge on plate *B* of pharynx.
Br, brain.
C, dorsal or wing plates of pharyngeal skeleton, developed in arms of dorsal pouch of atrium.
c, ridge on plate *C* of pharyngeal skeleton.
D, bridge plate of pharyngeal skeleton in roof of atrium.
d, vertical, longitudinal folds of floor of pharynx.
DiMcl, dilator muscles of pharynx.
DP, dorsal pouch of atrium, divided beyond base into two wings containing plates *C* of pharyngeal skeleton and leading to roots of frontal sacs.
DPMcl, dorsal protractor muscles of pharynx.
DPhy, dorsal wall of pharynx.
e, chitinous bridge in floor of atrium connecting pharyngeal plates *A*.
EMcl, extensor muscles of oral hooks.
f, two small sclerites in floor of pharynx just behind the mouth.
FMcl, flexor muscle of oral hook.
FS, frontal sacs, containing imaginal buds of antennæ and compound eyes.
G, pupal hypoderm separated from larval pharyngeal skeleton. (See *A*.)
g, anterior sense papilla on snout of larval head.
GC, gastric caecum.
Gng, ventral ganglionic nerve mass.
h, posterior sense papilla of snout of larval head.
Hk, *Hks*, oral hook, oral hooks.
L_1, L_2, L_3, leg buds of prothoracic, mesothoracic, and metathoracic legs, respectively.
LbB, imaginal buds of labium.
LH, larval head.
LPMcl, lateral protractor muscles of pharynx.
Mth, larval mouth.
op, optic bud, imaginal bud of compound eye.
Œ, œsophagus.
Phy, lumen of larval pharynx.
Pvent, proventriculus.
SalD, salivary duct.
SalGl, salivary gland.
Vent, ventriculus.
VPhy, ventral wall of pharynx.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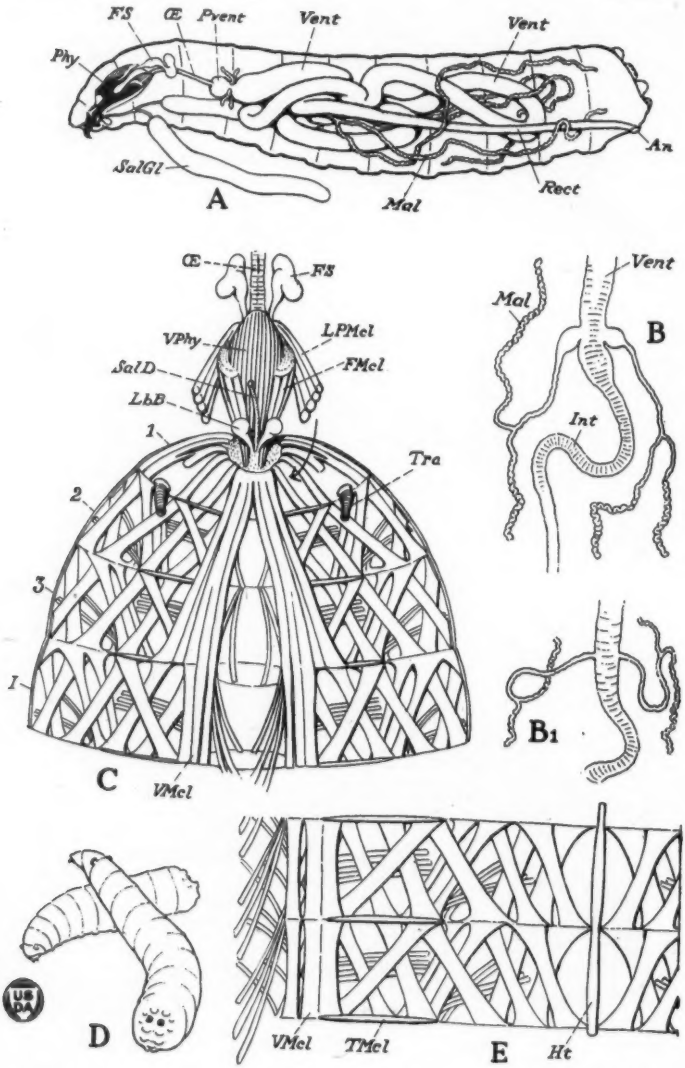
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PLATE 4

Rhagoletis pomonella

A.—Outline of mature maggot, showing position of larval pharynx, alimentary canal, and salivary glands.

B.—Union of stomach and intestine of larva, showing bases of Malpighian tubules.

B_1.—The same parts in the adult.

C.—Musculature of ventral and lateral parts of first four segments of mature larva, showing pharynx turned forward and the lateral pharyngeal protractor muscles separated from attachments on body wall as indicated by arrow on right. The roots of salivary duct and labial buds seen below anterior end of pharynx.

D.—Maggots in attitudes of extension and retraction of anterior segments.

E.—Musculature on right side and back of two consecutive segments, more enlarged than in C, but in corresponding position.

EXPLANATION OF SYMBOLS

1, prothorax.
2, mesothorax.
3, metathorax.
I, first abdominal segment.
An, anus.
FMcl, flexor muscle of oral hook.
FS, frontal sacs, containing imaginal buds of antennæ and compound eyes.
Ht, heart.
Int, intestine.
LbB, imaginal buds of labium.
LPMcl, lateral protractor muscles of pharynx.
Mal, Malpighian tubules.
Œ, œsophagus.
Pvent, proventriculus.
Phy, larval pharyngeal skeleton.
Rect, rectum.
SalD, salivary duct.
SalGl, salivary gland.
TMcl, transverse, intersegmental muscles.
Vent, ventriculus.
VMcl, latero-ventral longitudinal muscles of body wall.
VPhy, ventral wall of pharynx.

PLATE 5

Rhagoletis pomonella

A.—Puparium, lateral view.

B.—Puparium, dorsal view.

C.—Prepupa, or fourth-instar larva, exposed inside the puparium, 24 hours after formation of puparium.

D.—Pupa in early part of first or cryptocephalic stage, shedding the prepupal cuticle over the thorax, 43 hours after formation of puparium.

E.—The same as D, ventral view, but with puparial shell and larval pharynx removed, exposing a deep hole (*Atr*) in anterior end of pupa.

F.—Final phase of cryptocephalic pupa (*Pu*) inclosed in two envelopes, the puparial shell (*pm*) and the prepupal skin (*ppu*) now shed over entire body, but with larval linings of stomodeum, proctodeum, and tracheæ not yet cast out of body of pupa.

EXPLANATION OF SYMBOLS

An, anus.

ASp, anterior larval spiracle.

Atr, atrium, anterior part of larval pharynx resulting from involution of original head of larva.

Mth, larval mouth.

N_1, pronotum.

N_2, Mesonotum.

o, horizontal cleavage line of puparium.

p, circular cleavage line of puparium.

pm, puparium.

ppu, prepupal larva (fourth larval instar, inside of puparium).

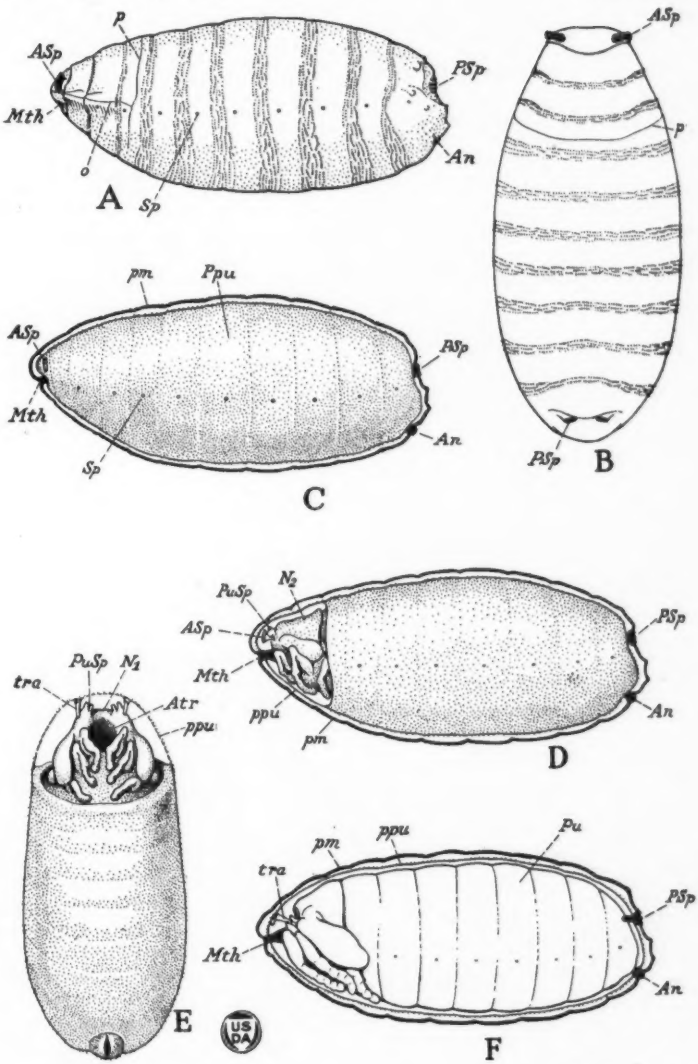
PSp, posterior larval spiracle.

Pu, pupa.

PuSp, pupal dorsal spiracle of pronotum.

Sp, lateral spiracles, the adult system, connected with lower longitudinal tracheal trunks.

tra, tracheal linings of preceding instar.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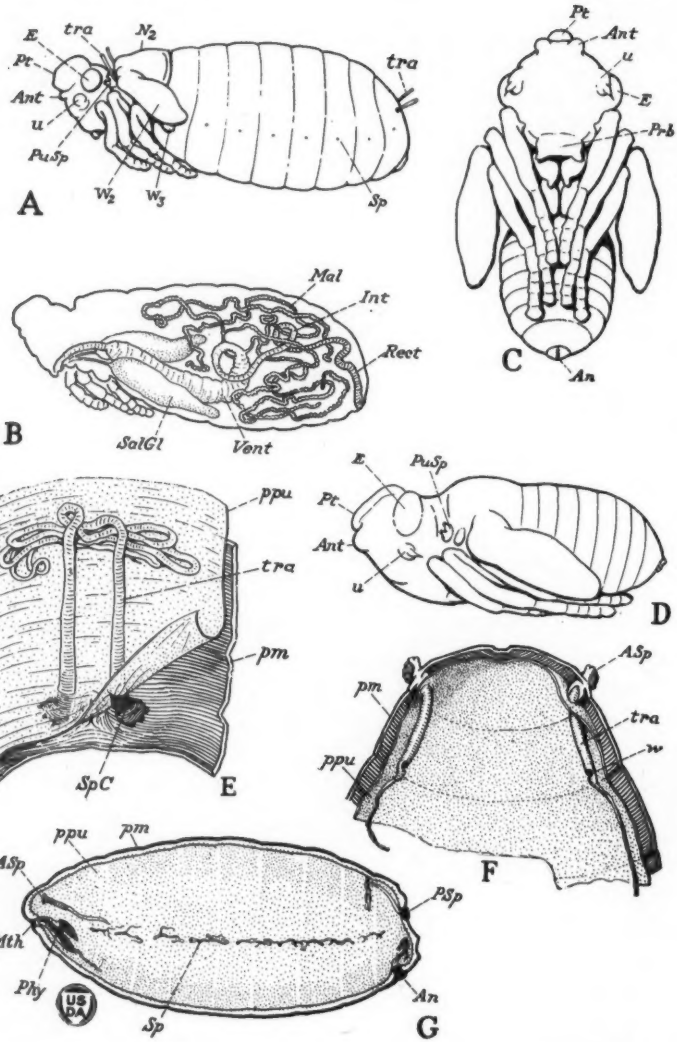
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PLATE 6

Rhagoletis pomonella

A.—Pupa in early part of second or phanerocephalic stage. Head everted, larval tracheal linings still retained.

B.—Pupal alimentary canal and salivary glands at period shown in A. (Compare with Plate 4, A.)

C.—Second phase of phanerocephalic pupa, the final pupal form, ventral aspect.

D.—Same as C, lateral aspect.

E.—Cast linings of dorsal tracheal trunks of larva connected with stigmatic chambers of posterior spiracles of puparium, showing usual arrangement against inner wall of molted prepupal cuticle enclosing the pupa.

F.—Inner surface of dorsal part of puparial cuticle through first three segments, and prepupal cuticular envelope of pupa, showing cast larval tracheal tubes still connected with anterior larval spiracles of puparium, and opening into pupal chamber by ruptures in second segment, thus establishing air passages to the pupa.

G.—Median, lengthwise section of puparium, and inner pupal envelope formed of cuticle of prepupal instar, pupa removed, showing cast linings of dorsal tracheal trunk connected with anterior and posterior larval spiracles of puparium, linings of lateral trunks and branches connected with lateral spiracles, and intima of pharynx and intestine connected with pupal envelopes at mouth and anus.

EXPLANATION OF SYMBOLS

An, anus.
Ant, antennal lobes.
ASp, anterior larval spiracle.
E, compound eye.
Int, intestine.
Mal, Malpighian tubules.
Mth, larval mouth.
N_2, mesonotum.
Phy, larval pharyngeal skeleton.
pm, puparium.
ppu, cuticle of prepupal larva.
PSp, posterior larval spiracle.
Prb, proboscis.
Pt, ptilinum.
PuSp, pupal dorsal spiracle of pronotum.
Rect, rectum.
SalGl, salivary gland.
Sp, lateral spiracles, the adult system, connected with lower longitudinal tracheal trunk.
SpC, stigmatic chamber.
tra, tracheal linings of preceding instar.
u, subocular lobe of pupa.
Vent, ventriculus.
W_2, wing; W_3, haltere.

CHEMICAL AND BIOLOGICAL STUDIES WITH CYANAMID AND SOME OF ITS TRANSFORMATION PRODUCTS[1]

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INTRODUCTION

In order for any nitrogenous material to be a satisfactory source of nitrogen for a wide variety of plants and under varying soil conditions it is necessary that the nitrogen be present either in the nitrate form or be readily attacked by the soil flora and converted into nitrates. While many plants can use ammonia and certain organic forms of nitrogen for their synthetic processes, most plants require at least a portion of their nitrogen in the form of nitrates. The investigations reported here were undertaken in order to obtain data on the rate at which cyanamid (calcium cyanamid) and its transformation products are broken down to ammonia and the latter oxidized to nitrates in the soil. In addition, the effect of dicyanodiamid and guanylurea on the nitrification of the soil organic matter or nitrogenous fertilizers which may be applied has been given consideration. The term ammonification is used here in the broader sense to include all of the decomposition processes from which ammonia is finally obtained. The term nitrification covers the oxidation of the ammonia to nitrates with nitrites as an intermediate product.

For convenience this paper has been divided into two parts; the first dealing with cyanamid and urea which under normal conditions are transformed into compounds which are suitable plant foods; the second with dicyanodiamid, guanylurea, guanidin, and biguanid nitrogen which are less desirable in soils. While it is probable that these latter compounds may serve as sources of nitrogen for plants in some instances, their value is questionable and some of them, particularly dicyanodiamid, are harmful.

In using the term transformation products of cyanamid to include the material discussed in Part II, it is not meant to imply that all of them are formed in the soil when cyanamid is applied. Whether or not this is true still remains to be shown. We do know that small amounts of dicyanodiamid are commonly formed and under unusually bad soil conditions the amount may be large, perhaps 25 per cent of the original nitrogen. There is little known as to the formation of salts of guanylurea, guanidin, and biguanid but it is logical to suppose that small percentages of these may appear under certain soil conditions. The uncertainty with regard to the appearance of these compounds does not necessarily detract from the value of a study of their behavior because they may be found in old samples of cyanamid or in fertilizer mixtures containing acid salts with which the cyanamid has reacted.

PREVIOUS INVESTIGATIONS AND DISCUSSION

CYANAMID AND UREA

A large number of laboratory and field studies have been reported which deal with the changes which cyanamid undergoes in the soil and the response of

[1] Received for publication, Jan. 4, 1924.

various crops to the products produced. However, only a few of these which throw light on the nature of the cyanamid changes, particularly those resulting from biological action, are mentioned here. The work is discussed approximately in the order reported.

Löhnis (*13*)[1] stated that on laboratory culture media cyanamid was converted into ammonia by bacterial action in the same manner as that of urea but at a slower rate. Dicyanodiamid was also formed in the absence of microorganisms by the action of water. Perotti (*22*) obtained results quite similar to those reported by Löhnis.

Kappen (*9*) found that in loam soil the transformation of cyanamid was very rapid and only small percentages of dicyanodiamid were formed. He attributed the first poisonous effects on plants following top dressings of cyanamid on acid soils to cyanamid, the later effects to dicyanodiamid.

Müntz and Nottin (*21*) obtained a lower rate of nitrification of calcium cyanamid than of ammonium sulphate when used in relatively large amounts but found little difference at the lower rates. They attributed the retardation to the cyanamid, itself, and not to the caustic lime present. Likewise, Lipman and Brown (*12*) found that cyanamid was injurious to nitrifying bacteria.

Löhnis and Sabaschnikoff (*16*) stated that cyanamid is not converted to any appreciable extent into ammonia in sterile soils but microorganisms are necessary for the change. Contrary to the opinion of Löhnis, Ulpiani (*26*, *27*) concluded from his studies that cyanamid is not converted into ammonia by bacteria, but easily changes into dicyanodiamid, urea, and other compounds. The dicyanodiamid and urea are both converted into ammonia. In a subsequent paper (*15*) Löhnis and Moll agreed with Ulpiani that there is no direct bacterial action on cyanamid, but that microorganisms are responsible for the formation of ammonia from the urea, first produced from cyanamid by soil colloids. On the other hand, Kappen (*10*) claimed to have found five species of fungi which readily attacked cyanamid.

Stutzer, Reis, and Söll (*25*) believed that the formation of ammonia from cyanamid was due chiefly to chemical action but, nevertheless, claimed that cyanamid but not dicyanodiamid can be utilized by microorganisms as a source of nitrogen. Brioux (*2*) found that in soil some cyanamid polymerizes into dicyanodiamid but that the latter is quickly converted into ammonia.

The importance of colloids was emphasized by several investigators, Henschel (*7*) having obtained a somewhat more rapid decomposition of cyanamid in dry sterilized soils and colloids than in the unsterilized. The presence of humus was of especial importance. Milo (*20*) studied the decomposition of cyanamid in soils and states that in heavy, strongly absorbent soils, high in colloidal matter urea is quickly formed by chemico-physical action. The urea is in turn changed to ammonium carbonate probably by microorganisms. In light soils the calcium cyanamid formed basic calcium cyanamid salts and free cyanamid. Ammonia was formed from these very slowly, thus giving time for dicyanodiamid formation. Löhnis (*14*) as a result of further studies, similar to those mentioned, agreed with Kappen that soil fungi are able to attack cyanamid directly but emphasized that normally cyanamid is changed into urea by means of colloids and that this is in turn changed into ammonia and nitrates by biological action.

Mazé and Lemoigne (*19*) found that a number of common soil bacteria are capable of converting cyanamid into urea. This change takes place rapidly in fertile soils, rich in humus, but slowly in acid soils. The urea is quickly changed to ammonia and nitrates.

Cowie (*3*) in a study of the chemistry of cyanamid changes in soil reported that cyanamid is readily changed to ammonia and nitrates in the absence of dicyanodiamid. When the latter compound is present ammonia accumulates

[1] Reference is made by number (italic) to "Literature cited," p. 68-69.

and nitrification is inhibited. In further work (*4*) with cyanamid he concluded that urea formation is the result of a purely chemical process and not biological. The breaking down of the urea is the result of the work of microorganisms. Cyanamid decomposition was more rapid in clay than in sandy soils.

The wide variations in the results reported by investigators who have studied cyanamid decomposition may be attributed largely to the differences in the methods of experimentation, including variations in the cyanamid and soils used. For instance, any experimentation with cyanamid containing appreciable quantities of dicyanodiamid or under conditions that favored its formation would undoubtedly greatly affect the conclusions reached by the investigator. This point will be considered more fully in the latter part of this report. However, the most recent investigations, as well as the observations of the writers, make it seem probable that under normal soil conditions cyanamid quickly changes into calcium acid cyanamid, then free cyanamid, urea, and ammonia. The ammonia is in turn oxidized to nitrites and nitrates. Dicyanodiamid is produced as a side reaction but only in small amounts, except under unfavorable soil conditions. This point has been considered in another publication (*1*) particularly in relation to the utilization of cyanamid by plants. The exact nature of the changes taking place, whether the result of chemical or biological action, is still disputed. However, it seems that the changes which result in the production of urea from cyanamid are largely, if not wholly, chemical and physical. Colloids are especially important in hastening these processes. The formation of ammonia from urea and its oxidation to nitrates is the result of the work of microorganisms.

DICYANODIAMID

In a study of dicyanodiamid De Grazia (*6*) failed to obtain nitrification. Perotti (*23*) studied the growth of bacteria on culture media containing dicyanodiamid and concluded that it is a good nitrogen food for many microorganisms. Ulpiani (*26*) reported that dicyanodiamid is slowly converted into ammonia. Loew (*17*) found that dicyanodiamid is not a good source of nitrogen for many kinds of soil bacteria. Löhnis (*15*) failed to secure any evidence that bacteria attack dicyanodiamid in either dilute or concentrated solutions. Stutzer (*25*) reported that dicyanodiamid can not be used by microorganisms, but in the soil is converted into ammonia by the chemical action of certain soil constituents. Reis (*24*) stated that certain microorganisms can use dicyanodiamid and also guanylurea and biguanid nitrogen in concentrations of less than one part per thousand of water. Brioux (*2*) found that if thoroughly mixed with the soil dicyanodiamid is quickly converted into ammonia and nitrified. Cowie (*3*) failed to obtain any evidence of nitrification of dicyanodiamid in soils even after several months. Furthermore, it was toxic to the nitrifying organisms and stopped the oxidation of ammonia in soils containing ammonium sulphate. It did not prevent the formation of ammonia from cyanamid or other forms of nitrogen but it did prevent the oxidation to nitrates, resulting in an ammonia accumulation. This extreme toxicity was limited to the nitrifying bacteria, the other soil organisms apparently being practically unaffected. Linter (*11*) did not find that any decomposition of dicyanodiamid occurred in soils during a period of several days.

The results of the investigations mentioned vary as to the readiness with which dicyanodiamid is attacked by bacteria. Where decomposition was obtained, some writers attribute it to purely chemical action, others to biological. No attempt was made in the work reported here to determine the agents responsible for the breaking down of the compound, but certainly there is no doubt but that the material does go over to ammonia. With the exception of the work of

Brioux, the previous investigations noted agree that dicyanodiamid does not nitrify. Using the ordinary applications of nitrogen and the short incubation period, the present work bears out this statement, but using 12.7 mgm. of nitrogen per 250 gm. of soil a nitrification of 38.6 per cent was obtained after 40 weeks. It is logical to suppose that nitrification should eventually take place since ammonia is formed fairly readily. The extreme toxicity of the dicyanodiamid for the nitrifying organisms prevents the normal oxidation process until most of the material has gone over to ammonia and the bacteria have recovered. It is this property of dicyanodiamid which is largely responsible for its injurious effect in fertilizers, but doubtless it is also directly toxic to many plants.

GUANYLUREA

The experimental work with guanylurea which has been reported is very limited and consists for the most part of plant culture studies. Since such studies are not directly related to the work here reported no references are given. It is sufficient to state that for the most part guanylurea has not served as a satisfactory plant food and in some cases has been reported as toxic. The behavior of the material in laboratory studies would lead us to expect just such a response. The material ammonified very slowly, and hence nitrate formation was limited. Where guanylurea sulphate was applied with ammonium sulphate the nitrification of the latter was inhibited for some time, but the toxic effect was decidedly less than with dicyanodiamid. However, such large quantities of gaunylurea sulphate were required to appreciably affect the oxidation of ammonium sulphate under laboratory conditions that it seems highly improbable that the material would ever be present in fertilizers in quantities sufficient to be injurious to field crops. Nitrogen present in the guanylurea form should be considered as inert material without fertilizer value.

GUANIDIN

Guanidin has given quite variable results when used as a fertilizer. In most cases the nitrate salt has been used and any increases in growth have been attributed to the nitrate rather than to the guanidin. Again, the nitrification experiments would lead us to expect the material to be an unsatisfactory source of nitrogen since it prevented nitrification for several weeks after application. Later it was quite rapidly converted into nitrates. If used on a soil containing sufficient nitrate nitrogen to supply the needs of the plants for the first four to eight weeks until the guanidin nitrogen had become available then the salts might act as good fertilizers. This also explains why in some cases guanidin salts gavé better results on the second crop than on the first.

BIGUANID

Laboratory or field studies with biguanid nitrogen are very limited and its value still remains to be established. Reis (*24*) found that in the laboratory the compound could be used by microorganisms if the concentration was sufficiently low. In the present nitrification studies a maximum conversion of 9.7 per cent was obtained after 50 days. This figure is so low that the slight increase in nitrates may have come from the soil organic matter rather than from the biguanid nitrogen. Again, the increase may have represented merely the injury which the biguanid caused to the organisms which use nitrate nitrogen.

PLAN OF THE WORK

The experiments, which consisted chiefly of ammonification and nitrification studies, were conducted in the laboratory, using either 100 or 250 gm. of air-dried

soil in 200 or 300 cc. beakers, to which were added the various materials being studied. The soil used for all of the experiments was a Susquehanna loam, well supplied with organic matter and quite productive. It had been in constant cultivation for about 15 years. As shown by the experimental results, it contained a sufficient supply of basic elements to show a high nitrifying efficiency even without additions of calcium carbonate.

In preparing mixtures, usually the required amount of the dry salt or fertilizer was thoroughly mixed with the soil sample by means of a small aluminum shaker of the type commonly used in soda fountains. In a few cases the soluble salts were added in solution. The moisture content was brought up to the optimum of 21 cc. per 100 gm. of soil, except in one instance where this was made the variable factor. The water lost by evaporation was replaced at intervals of four or five days. During the incubation period the soils were maintained at room temperature, usually about 20° to 22° C., except where the temperature was made the variable factor.

The analyses of the nitrogenous compounds used is given in Table I. The untreated cyanamid was used unless otherwise stated.

ANALYTICAL METHODS

DETERMINATION OF AMMONIA NITROGEN

To a 25-gm. sample of soil were added 50 cc. of a solution of sodium carbonate and sodium chlorid (108 gm. Na_2CO_3 and 150 gm. NaCl per liter) and about 1 cc. of paraffin oil. The mixture was then aerated for six hours in the apparatus devised by Matthews (*18*). The ammonia evolved was absorbed in N/20 sulphuric acid and the excess titrated with N/20 sodium hydroxid, using methyl red as an indicator.

TABLE I.—*Total nitrogen content of materials used*

	Total nitrogen (per cent)		Total nitrogen (per cent)
Cyanamid, hydrated and oiled	18. 32	Guanylurea sulphate	34. 92
Cyanamid, untreated	20. 61	Guanidin nitrate	45. 44
Urea	46. 44	Guanidin carbonate	46. 62
Ammonium sulphate	20. 90	Nitro guanidin	53. 84
Dicyanodiamid	66. 31	Biguanid nitrate	51. 00

TABLE II.—*Determination of ammonia by the aeration method*

Material	Mgm. N added	Nitrogen recovered as ammonia							
		1.5 hours		3 hours		5 hours		6 hours	
		NH_3 mgm. N	Average	NH_3 mgm. N	Average	NH_3 mgm. N	Average	NH_3 mgm. N	Average
Blank	0. 00			0. 04				0. 04	
Soil	.00			.19		0. 19		.19	
	.00			.17	0. 18	.18	0. 18	.21	0. 20
Ammonium sulphate from solution	10. 40	9. 07		10. 37				10. 38	
	10. 40	9. 73	9. 40	10. 40	10. 38			10. 38	10. 38
Ammonium sulphate from soil	10. 40			9. 64		10. 21		10. 27	
	10. 40			9. 89	9. 76	10. 17	10. 19	10. 22	10. 24
Dicyanodiamid	2. 54							.005	

The accuracy of the method is indicated by the results of the preliminary experiments reported in Table II. Almost complete recovery of the ammonia in ammonium sulphate was secured after aerating three hours. In the presence of soil it was more difficult to drive off the ammonia, 93.9 per cent being recovered in three hours and 98.5 per cent after six hours. When a dicyanodiamid solution was aerated the recovery as ammonia was 0.18 per cent. This small amount is easily within experimental error when dealing with such small quantities of nitrogen.

DETERMINATION OF NITRATE NITROGEN

Two methods of determining nitrates were used in this work, namely, the reduction and the colorimetric methods.

REDUCTION METHOD

The determination of nitrate nitrogen by the reduction method, using Devarda's alloy, is now generally considered as the most satisfactory for most purposes. However, in the presence of undecomposed cyanamid, dicyanodiamid, urea, or guanylurea the reduction method as ordinarily carried out gives high results. This is because these compounds, when boiled with weak alkali, slowly decompose with the evolution of ammonia. A modification of the method to avoid the difficulties was devised and has been reported elsewhere (*8*). The modified procedure was used in this work wherever the reduction method was employed.

COLORIMETRIC METHOD

The phenol-disulphonic acid method of determining nitrates in soil solutions has been practically set aside in recent years because there are numerous salts and organic materials which interfere in the color production or particular shade of color. Regardless of these objections to an indiscriminate use of the method it is nevertheless recognized that under certain conditions the method is quite accurate, and much shorter than the reduction method.

A comparison between the modified reduction method and the phenol-disulphonic acid method was made during the course of these investigations on samples of soil containing cyanamid and its decomposition products. For the particular soil used the results were in quite close agreement and the compounds which require elimination in the case of the reduction method did not interfere in the colorimetric method. For small quantities of nitrate nitrogen the latter method is more accurate, and since it is shorter it was used in obtaining a portion of the results reported in this paper. Where this method was used the fact is mentioned, otherwise the modified reduction method was used.

DETERMINATION OF COMBINED CYANAMID AND DICYANODIAMID NITROGEN

The method of Brioux was used for the determination of cyanamid and dicyanodiamid nitrogen. To 50 cc. aliquots of soil extract were added 10 cc. each of 5 and 10 per cent solutions of silver nitrate and potassium hydroxid, respectively. It was found necessary to wash the precipitates 12 or 13 times with distilled water in order to remove all traces of ammonia. The total nitrogen in the precipitate was then determined by the usual Kjeldahl method.

DETERMINATION OF UREA NITROGEN

The short urease method as developed at this laboratory by Fox and Geldard (*5*) was used for the determination of urea. This consists in adding to a 50 cc.

aliquot of soil extract 10 cc. of a freshly prepared neutral 2 per cent water extract of Jack-bean flour. After standing for one hour at room temperature the solution is made acid with a measured excess of standard acid, aerated for 5 minutes to drive off carbon dioxid and titrated. The acid used up is a measure of the ammonia formed from the urea. Blanks on soil extract alone showed no interference in this method.

EXPERIMENTAL RESULTS

PART I.—AMMONIFICATION AND NITRIFICATION OF CYANAMID AND UREA

COMPARATIVE RATES OF NITRIFICATION OF CYANAMID, UREA, AND AMMONIUM SULPHATE

The soil used for these experiments was obtained fresh, then sieved and air-dried. An analysis of the dry soil showed 0.56 mgm. of nitrate nitrogen per

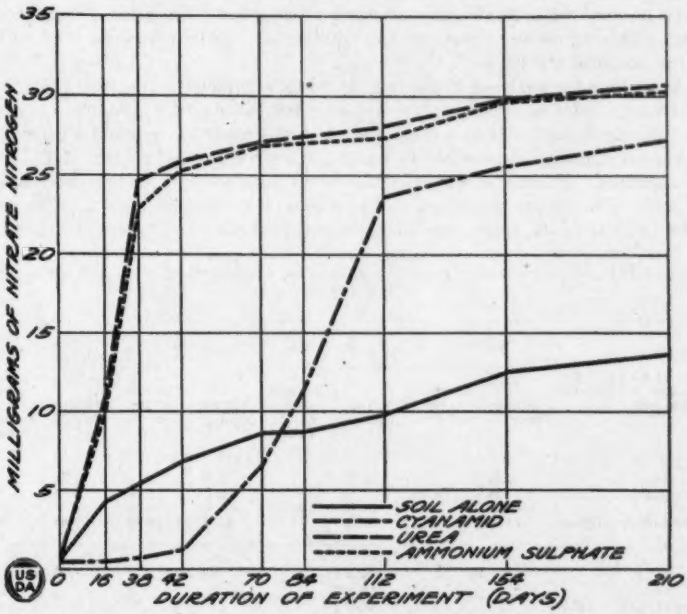

FIG. 1.—Diagram showing the rates of nitrification of cyanamid, urea, and ammonium sulphate.

100 gm. of soil. One gm. of powdered calcium carbonate was added to each 100 gm. of soil in order to insure neutrality. The figures given in Table III are the averages of duplicate determinations.

Considering the results in Table III, which are shown diagrammatically in figure 1, it will be observed that there was no very great difference between the rates of nitrification of ammonium sulphate and urea, the slight difference being in favor of the latter material. The maximum conversion of 87.8 per cent for ammonium sulphate was reached in 42 days, while with urea the maximum, 91.9 per cent, was reached in 28 days.

Cyanamid failed to show any nitrification during the first 70 days but actually depressed the nitrate formation from the soil organic matter. At the end of 84 days there was a 12.77 per cent conversion and after 112 days the maximum of only 66.65 per cent was reached. From subsequent experiments (Table IV) with the same or larger quantities of cyanamid it appears probable that the presence of the calcium carbonate had a deleterious effect upon the rate of nitrification of this material.

AMMONIFICATION AND NITRIFICATION OF VARYING QUANTITIES OF TREATED AND UNTREATED CYANAMID

These experiments were made to determine whether hydrating and oiling cyanamid has any effect upon its rate of decomposition and nitrification and hence upon its value as a fertilizer. The treatment given the cyanamid was the same as ordinarily used for commercial cyanamid and consisted of hydration with 8 per cent water and the addition of 3.5 per cent of mineral oil.

It was also desired to determine the maximum quantity of cyanamid which may be used without seriously affecting nitrification. Since the soil showed a high nitrifying efficiency without the addition of calcium carbonate none of the latter material was used.

In making the analyses, if urea was found to be present two samples of soil were analyzed, one for ammonia, nitrate, and urea and the other for combined cyanamid and dicyanodiamid. Only a qualitative test was made for cyanamid because of the great difficulty experienced in washing the silver precipitate free of all traces of ammonia. When urea was absent all of the analyses were made on one sample of soil. The results are given in Table IV and in part in figure 2. The data used in plotting the graph were those secured with oiled and hydrated cyanamid.

TABLE III.—*Comparison of rates of nitrification of cyanamid, urea, and ammonium sulphate*

Treatment (20.9 mgm. N per 100 gm. soil)	16 days		28 days		42 days		70 days	
	Nitrate mgm. N	Increase over control mgm.	Nitrate mgm. N	Increase over control mgm.	Nitrate mgm. N	Increase over control mgm.	Nitrate mgm. N	Increase over control mgm.
Soil alone	4.31		5.51		6.91		8.65	
Cyanamid	[a].48	0.00	.74	0.00	1.23	0.00	6.70	0.00
Urea	11.31	7.00	24.71	19.20	25.86	18.95	27.13	18.48
Ammonium sulphate	10.64	6.33	23.01	17.50	25.26	18.35	26.88	18.23

Treatment (20.9 mgm. N per 100 gm. soil)	84 days		112 days		154 days		210 days	
	Nitrate mgm. N	Increase over control mgm.	Nitrate mgm. N	Increase over control mgm.	Nitrate mgm. N	Increase over control mgm.	Nitrate mgm. N	Increase over control mgm.
Soil alone	8.66		9.83		12.50		13.75	
Cyanamid	11.33	2.67	23.76	13.93	25.58	13.08	27.28	13.53
Urea			28.11	18.28	29.85	17.35	30.78	17.03
Ammonium sulphate			27.39	17.56	29.65	17.15	30.27	16.52

[a] Qualitative tests showed absence of cyanamid nitrogen.

The ammonia and nitrate production from cyanamid, particularly nitrate, was dependent upon the rate of application. With the smallest applications nitrification was complete within 19 days while with the largest amount, namely, 104.4 mgm. N per 250 gm. of soil, nitrification was only about 50 per cent complete in 200 days. In such cases the cyanamid nitrogen remained in the soil largely in the form of cyanamid, dicyanodiamid, and ammonia. In the case of the small applications of cyanamid more than a 100 per cent recovery of the

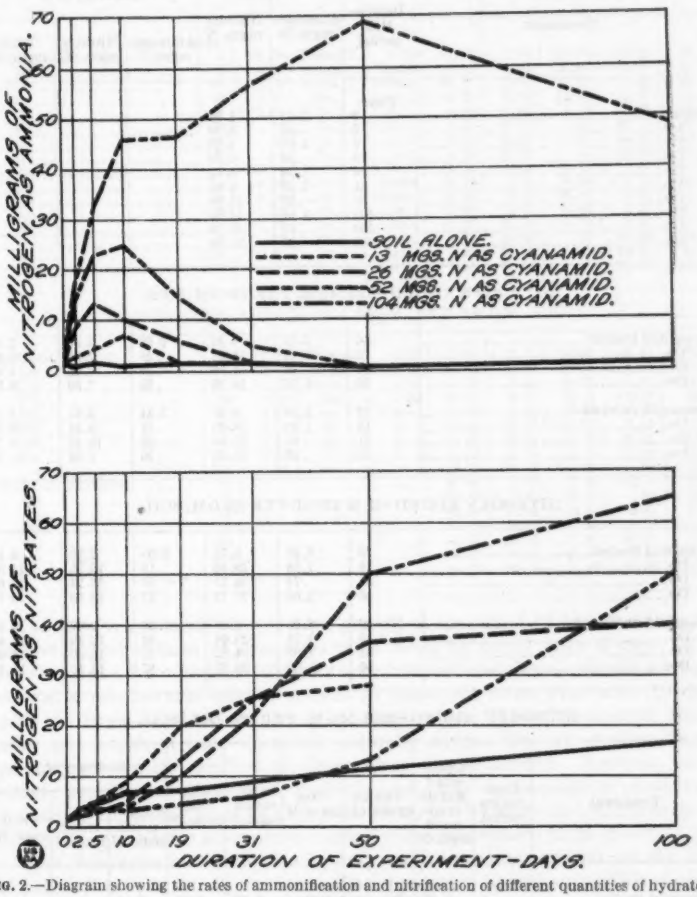

FIG. 2.—Diagram showing the rates of ammonification and nitrification of different quantities of hydrated and oiled cyanamid

added nitrogen was obtained in some instances. This may have been due to a stimulating action which small quantities of cyanamid may possibly have had upon the nitrifying organisms, causing a more rapid nitrification of the organic soil nitrogen than takes place in the soil containing no cyanamid. However, no great significance should be attached to this since, as previously stated, the reduction method is not very accurate for the determination of very small amounts of nitrates. In these experiments 200 gm. of soil were extracted with 500 cc. of

water and 200 cc. aliquots distilled. Each distillation, therefore, represented only 2 to 3 mgm. of the original cyanamid nitrogen in the case of the smallest applications.

TABLE IV.—*Ammonification and nitrification of varying quantities of treated and untreated cyanamid*

Treatment	Incubation period	Ammonia mgm. N	Nitrates mgm. N	Recovery of N		
				Ammonia mgm. N	Nitrates mgm. N	Total mgm. N
	Days					
Soil alone	0	1.52	1.64			
Do	2	.08	3.39			
Do	5	1.07	4.55			
Do	10	.84	6.36			
Do	19	1.20	6.72			
Do	31	1.33	8.53			
Do	50	.65	11.36			
Do	100	1.21	16.01			
Do	150	.79	19.29			
Do	200	.26	20.78			
NITROGEN ADDED—6.52 MGM. PER 250 GM. SOIL						
Cyanamid treated	10	1.52	9.32	0.68	2.96	3.64
Do	19	1.48	14.38	.28	7.66	7.94
Do	31	.74	16.16	−.39	7.63	7.04
Do	50	1.15	19.10	.50	7.80	8.30
Cyanamid untreated	10	3.18	9.17	2.34	2.81	5.15
Do	19	1.21	16.06	.01	9.34	9.35
Do	31	.65	18.93	−.68	10.40	9.72
Do	50	.85	21.35	.20	9.99	10.19
NITROGEN ADDED—13.04 MGM. PER 250 GM. SOIL.						
Cyanamid treated	10	6.89	8.75	6.05	2.39	8.44
Do	19	1.38	19.10	.18	12.38	12.56
Do	31	.74	24.77	−.59	16.24	15.65
Do	50	1.00	27.72	.35	16.36	16.71
Cyanamid untreated	10	8.22	8.83	7.38	2.47	9.85
Do	19	1.34	20.04	.14	13.32	13.46
Do	31	1.02	24.83	−.31	16.30	15.99
Do	50	1.02	26.51	.37	15.15	15.52

NITROGEN ADDED—26.08 MGM. PER 250 GM. SOIL

Treatment	Incubation period	Cyanamid and dicyanamid mgm. N	Test for cyanamid	Urea mgm. N	Ammonia mgm. N	Nitrates mgm. N	Recovery of N		
							Ammonia mgm. N	Nitrates mgm. N	Total mgm. N
	Days								
Cyanamid treated	2	8.48	Present	0.37	8.22	2.26	7.42	−1.13	15.14
Do	5	1.87	None	None	13.18	2.61	12.11	−1.94	12.04
Do	10	.89	do	do	10.47	4.72	9.63	−1.64	8.88
Do	19	.80	do	do	6.52	12.26	5.32	5.54	11.66
Do	31	.59	do	do	1.39	24.77	.06	16.24	16.89
Do	50	.56	do	do	.32	36.27	−.33	24.91	25.14
Do	100	.00	do	do	1.21	40.03	.00	24.02	24.02
Cyanamid untreated	10				12.90	8.85	12.06	2.49	14.55
Do	19				4.23	21.32	3.03	14.60	17.63
Do	31				1.02	34.45	−.31	25.92	25.61
Do	50				1.10	38.21	.45	26.85	27.30
Do	100				1.21	43.75	.00	27.74	27.74

TABLE IV.—*Ammonification and nitrification of varying quantities of treated and untreated cyanamid*—Continued

NITROGEN ADDED—52.2 MGM. PER 250 GM. SOIL

Treatment	Incubation period	Cyanamid and dicyanamid mgm. N	Test for cyanamid	Urea mgm. N	Ammonia mgm. N	Nitrates mgm. N	Recovery of N		
							Ammonia mgm. N	Nitrates mgm. N	Total mgm. N
	Days								
Cyanamid treated	2	24.76	Present	1.73	13.86	2.38	13.06	−1.01	38.54
Do	5	8.23	Trace	Trace	22.12	2.53	21.05	−2.02	27.26
Do	10	3.75	None	None	24.42	3.94	23.58	−2.42	24.91
Do	19	2.49	do	do	14.10	9.89	12.90	2.87	18.26
Do	31	2.77	do	do	4.96	21.43	3.63	12.90	19.30
Do	50	.75	do	do	1.21	49.07	.56	37.71	39.02
Do	100	.00	do	do	1.22	64.35	.01	48.34	48.35
Do	150	.00	do	do	1.00	63.75	.21	44.46	44.67
Cyanamid untreated	2	23.14	Present	1.59	14.28	2.43	13.48	−.96	37.25
Do	5	6.48	None	Trace	25.75	2.36	24.68	−2.19	28.97
Do	10	3.13	do	None	27.40	3.70	26.56	−2.66	27.03
Do	19	.62	do	do	12.63	12.41	11.43	5.69	17.74
Do	31	1.28	do	do	11.03	21.10	9.70	12.57	23.55
Do	50	.72	do	do	.70	54.65	.05	43.29	44.06
Do	100	.00	do	do	1.23	64.52	−.02	48.51	48.53
Do	150	.00	do	do	1.26	66.69	.47	47.40	47.87

NITROGEN ADDED—104.4 MGM. PER 250 GM. SOIL

Cyanamid treated	2	57.92	Present	5.59	17.68	2.95	16.88	−0.44	79.95
Do	5	32.77	do	1.47	31.63	2.80	30.56	−1.75	63.05
Do	10	14.53	None	1.11	45.62	3.02	44.78	−3.34	57.08
Do	19	9.84	do	1.20	46.35	4.21	45.15	−2.51	53.68
Do	31	9.37	do	None	56.60	5.58	55.27	−2.95	61.69
Do	50	9.04	do	do	68.43	13.17	67.78	1.81	78.63
Do	100	3.61	do	do	48.44	49.94	47.25	33.93	84.77
Do	150	4.20	do	do	41.87	56.78	41.08	37.49	82.77
Do	200	2.65	do	do	39.52	69.47	39.26	48.69	90.60
Cyanamid untreated	10				51.08	3.62	50.24	−2.74	47.50
Do	19				39.25	7.67	38.05	.95	39.00
Do	31				60.20	11.36	58.87	2.83	61.70
Do	50				41.78	28.48	41.13	17.12	58.25
Do	100				25.07	59.92	23.86	43.91	67.77
Do	150				42.93	54.91	42.14	35.62	77.76
Do	200				17.68	85.78	17.42	65.00	82.42

The test for cyanamid showed none present at the end of 10 days and with the smaller concentrations of cyanamid none could be found after 5 days. The figures for the determination of combined cyanamid and dicyanodiamid nitrogen showed a considerable amount present in these two forms even after 10 days with the larger applications. Since qualitative tests showed cyanamid absent after this length of time we would naturally assume that all of the nitrogen precipitated by the method of Brioux was dicyanodiamid. However, silver nitrate precipitates guanylurea, guanidin and other decomposition products of cyanamid which may possibly have been present. Whether such compounds are formed in appreciable amounts in normal soils still remains to be shown.

Urea determinations showed only very small quantities present at any time although it is undoubtedly an intermediate product in the decomposition of cyanamid in the soil. Ammonia formation from urea in the presence of a mixed flora of microorganisms takes place so rapidly that no accumulation can occur.

The two samples of cyanamid, one oiled and hydrated and the other untreated, nitrified at about the same rates, where used in the smaller concentrations. At the heavier rates the latter appeared to be somewhat better than the former. Shortly after these experiments were started the hydrated and oiled cyanamid used in the mixtures was again analyzed for its various forms of nitrogen. It was found that the dicyanodiamid nitrogen had increased considerably over that found at the previous analysis, which was made about two months before the

experiments were started. The better results obtained with the untreated as compared with the maximum hydrated and oiled material may be in part attributed to the presence of this dicyanodiamid in the latter. At no time could any dicyanodiamid be found in the original sample of untreated cyanamid.

THE DECOMPOSITION OF UREA IN SOIL

This experiment was conducted in order to obtain data in addition to that already given in Table III on the rate of ammonification and nitrification of urea. The procedure followed was the same as in the earlier experiment except that no calcium carbonate was used. Preliminary experiments on the recovery of known quantities of urea from soil immediately after the addition gave an average of 97.63 per cent. The failure to recover all of the added urea may have been due to the rapidity with which this compound is decomposed to ammonia

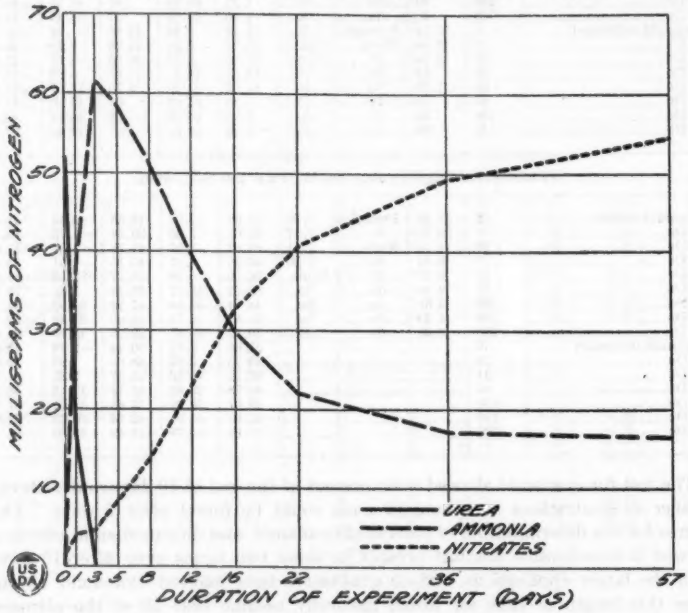

FIG. 3.—Diagram showing the rates of ammonification and nitrification of urea

in the soil. Further experiments to determine if urea is broken up in the aeration method for ammonia gave negative results.

The experimental results are given in Table V and figure 3. The results given for ammonia and nitrate nitrogen are the average of two determinations while those for urea are the average of three analyses, all made on the same sample of soil.

The conversion of urea into ammonia took place very rapidly, 65 per cent of the urea having disappeared within 24 hours while at the end of 3 days no trace of undecomposed urea could be found. A comparison of the results given in Table V with those in Table III shows a maximum nitrification of 74.6 per cent after 57 days in the former case and 91.8 per cent in 28 days in the latter. This wide variation is due to the marked stimulation of nitrate formation by the presence of calcium carbonate. It will be remembered that exactly opposite results were obtained in the case of cyanamid.

The importance of the manner in which the percentage nitrification is determined needs to be emphasized. There is no satisfactory method of measuring the actual per cent of the added nitrogen converted into nitrates in soil containing a mixed biological flora. All that is done is to determine the nitrates which accumulate over any given period. While the agents of decomposition are converting the nitrogen to ammonia, which in turn is oxidized to nitrites and nitrates, there are also numerous other organisms which are using the nitrates for their own growth. In this case the nitrates are usually converted into organic forms but under certain conditions may be merely denitrified. The soil conditions are, therefore, extremely important in determining the maximum nitrate accumulation at any given time. The more favorable the conditions for nitrate formation the quicker the maximum nitrate accumlation and usually the higher the figures. This is due chiefly to the fact that less time is available for the organisms, other than nitrifiers, to utilize the nitrate nitrogen. Where conditions permit only a slow rate of nitrification the nitrates may be utilized almost as fast as formed. The nitrogen thus converted into the organic form is again nitrified when the organisms die. Finally, an equilibrium point is reached after a long period where nitrate formation and nitrate utilization practically balance under a constant set of conditions. The nitrate nitrogen present at such a point is considerably lower than the maximum nitrate accumulation.

TABLE V.—*Ammonification and nitrification of urea*

Incubation period	Soil alone 250 gm.		Urea 52.25 mgm. N per 250 gm. soil					
						Recovery of N		
	Ammonia mgm. N	Nitrates mgm. N	Urea mgm. N	Ammonia mgm. N	Nitrates mgm. N	Ammonia mgm. N	Nitrates mgm. N	Total, including urea mgm. N
Days								
0	1.88	1.63	52.25	1.88	1.63			
1	3.40	2.40	17.92	37.73	4.08	34.33	1.68	53.93
3	3.81	3.80	.00	61.81	4.73	58.00	.93	58.93
5	3.26	5.43	.00	58.70	8.23	55.44	2.80	58.24
8	2.81	7.85	.00	51.54	13.82	48.73	5.97	54.70
12	1.88	10.01	.00	40.02	23.39	38.14	13.38	51.52
16	.11	9.26	.00	29.61	32.53	29.50	23.27	52.77
22	.47	10.09	.00	22.31	40.65	21.84	30.56	52.40
36	1.12	12.08	.00	17.37	49.00	16.25	36.92	53.17
57	1.30	15.57	.00	16.53	54.55	15.23	38.98	54.21

The necessity for having a neutral or basic soil reaction in order to secure a rapid formation and accumulation of nitrates is very important in this connection. As nitric acid is formed, available basic elements must be present for the formation of neutral salts; otherwise further nitrification is practically prohibited. At the same time the growth of soil fungi, which prefer an acid medium, is favored and these organisms help further to decrease the nitrate supply. The failure of calcium carbonate to favor the nitrification of cyanamid, as previously mentioned, is an exception, but this is thought to be due to the increase in dicyanodiamid under such conditions.

EFFECT OF MOISTURE UPON THE NITRIFICATION OF CYANAMID AND UREA

The use of cyanamid as a fertilizer under field conditions has shown quite variable results apparently due largely to seasonal conditions in many instances. For instance, it has been stated that a wet period in the early summer retards the nitrification of cyanamid and hence poor responses from the use of the material are secured. Again, it has been claimed that cyanamid should be applied only

during the cool months of the year since a hot summer, which is usually accompanied by a drought, favors the formation of dicyanodiamid rather than nitrates. In order to secure more accurate data upon these points the two experiments which follow were made.

The methods were the same as previously given except that only 100-gm. samples of soil were used. Urea was selected as the standard for comparison

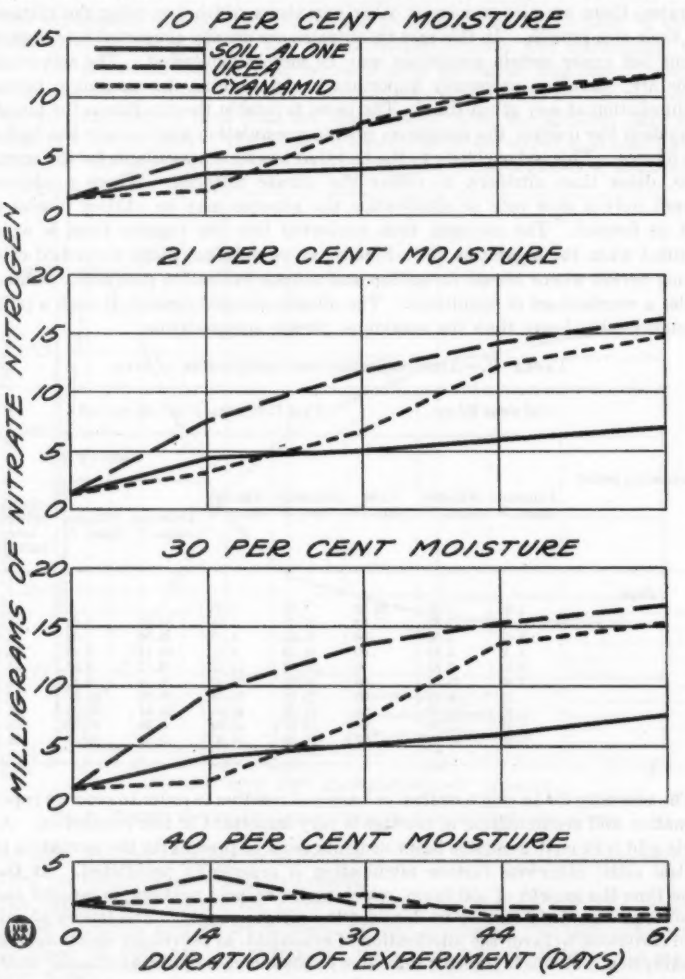

FIG. 4.—Diagram showing the rate of nitrification of cyanamid and urea in presence of different percentages of moisture

instead of ammonium sulphate. The soils were kept at room temperature during the incubation period. The data obtained by the colorimetric method, are given in Table VI and figure 4.

It will be noted that the per cent nitrification of cyanamid in the soil containing 10 per cent moisture is as high as that of urea under similar conditions

with the exception of the first incubation periods. This initial retardation is characteristic of cyanamid and occurs even under the most favorable conditions. In the presence of 21 and 30 per cent of water urea nitrified more rapidly in all cases than did cyanamid and even at the end of two months the nitrate accumulation was still higher in the case of urea. It should be noted that nitrification was the highest with both materials in the presence of 30 per cent of moisture which corresponds to nearly three-fourths of saturation. At half saturation (21 per cent), which is usually considered the optimum, the nitrate accumulation was appreciably below the figures for 30 per cent moisture. At 40 per cent moisture, which is near the saturation point, nitrification was negligible. In fact, there was usually a decrease of nitrates with an increase in the length of the incubation period. This may have been either the result of denitrification or utilization of the nitrates by microorganisms.

TABLE VI.—*Nitrate formation from cyanamid and urea in soils containing different percentages of moisture*

Incubation period	Soil alone nitrates mgm. N	Urea 10 mgm. N per 100 gm. soil—		Cyanamid 10 mgm. N per 100 gm. soil—	
		Nitrates mgm. N	Recovery of N as nitrates mgm. N	Nitrates mgm. N	Recovery of N as nitrates mgm. N
Days					
10 PER CENT MOISTURE					
14	3.57	5.15	1.58	2.53	−1.04
30	4.00	7.95	3.95	7.73	3.73
44	4.47	10.17	5.70	10.63	6.16
61	4.38	11.94	7.56	12.07	7.69
21 PER CENT MOISTURE					
14	4.65	7.72	3.07	3.50	−1.15
30	5.27	11.94	6.67	6.87	1.60
44	6.21	14.37	8.16	12.27	6.06
61	7.37	16.21	8.84	14.77	7.40
30 PER CENT MOISTURE					
14	4.16	9.54	5.38	2.23	−1.93
30	5.25	13.61	8.36	7.35	2.10
44	6.00	15.95	9.95	13.61	7.61
61	7.73	17.16	9.43	15.60	7.87
40 PER CENT MOISTURE					
14	0.38	3.81	3.43	(a)	(a)
30	.27	1.21	.94	2.42	2.15
44	.12	1.29	1.17	.35	.23
61	.00	1.08	1.08	.68	.68

[a] Lost.

EFFECT OF TEMPERATURE UPON THE NITRIFICATION OF CYANAMID AND UREA

This study of the nitrification of cyanamid was made according to the usual methods, using four different temperatures. One set of samples was kept in the open at a temperature varying from −9 to 18° C. Another series was maintained at a room temperature of 15 to 20° C. The remaining two sets of samples were kept at constant temperatures of 30° and 38.5° C. in incubators. The nitrate determinations, which were made by the colorimetric method, are given in Table VII. These data are plotted in figure 5.

The rate of nitrate formation at the lowest temperature was exceedingly slow in the case of the soil alone and also in the presence of urea. Even after 42 days, only about 10 per cent of the added nitrogen was recovered as nitrate nitrogen. With the equivalent application of cyanamid no nitrification occurred and even the nitrification of the soil organic matter was prevented. At room temperature

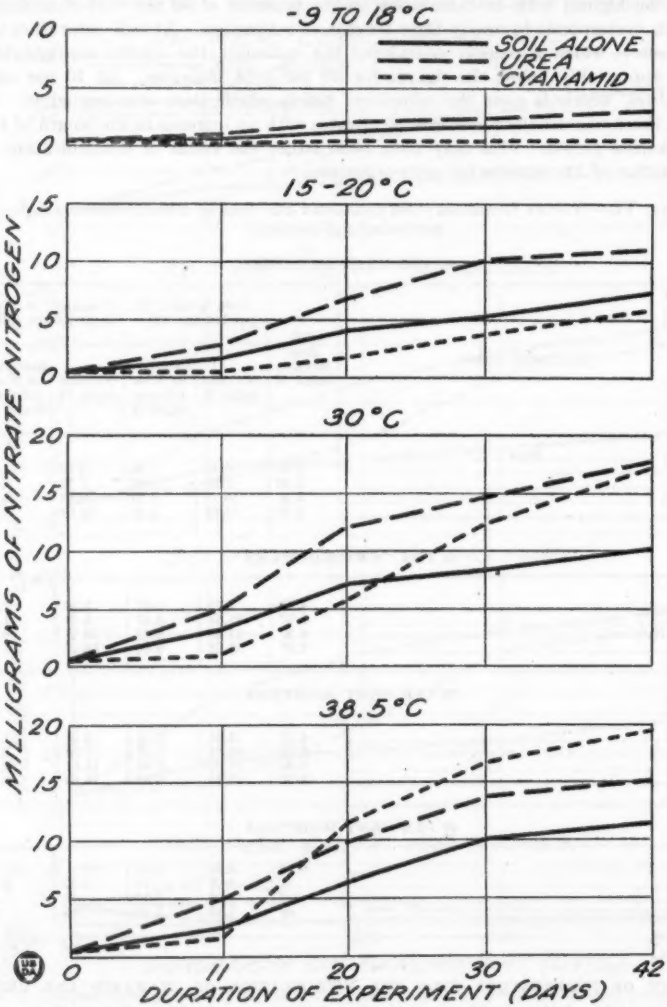

FIG. 5.—Diagram showing the rate of nitrification of cyanamid and urea at different temperatures

the urea nitrified to the extent of 49 per cent after one month but even after 42 days none of the cyanamid nitrogen was recovered as nitrate. In this experiment the same sample of cyanamid and the same soil type was used as in the previous experiments but the nitrification was poor. This may be attributed to the difference in the room temperature during the winter and summer months.

Nitrification of urea was the highest at 30° C., at which temperature 74 per cent of the added nitrogen was found as nitrate nitrogen. Under similar conditions cyanamid showed 69 per cent nitrification. At this temperature cyanamid retarded nitrification for 20 days but later was oxidized quite rapidly. At the highest temperature of 38.5° C. the nitrate accumulation from urea was considerably less than at 30° C.; but in the case of cyanamid was exceptionally good. The maximum nitrate accumulation for the entire experiment, which was 79.6 per cent, was obtained at this temperature. Under the same conditions urea gave 36.7 per cent.

TABLE VII.—*Nitrate formation from cyanamid and urea in soils maintained at different temperatures*

Incubation period	Soil alone nitrates mgm. N	Urea 10 mgm. N per 100 gm. soil—		Cyanamid 10 mgm. N per 100 gm. soil—	
		Nitrates mgm. N	Recovery of N as nitrates mgm. N	Nitrates mgm. N	Recovery of N as nitrates mgm. N
Days					
−9 TO 18° C.					
11	0.69	0.95	0.26	0.37	−0.32
20	1.36	2.06	.70	.50	−.86
30	1.56	2.40	.84	.54	−1.02
42	2.37	3.38	1.01	.73	−1.64
15 TO 20° C.					
11	1.84	2.72	0.88	0.55	−1.29
20	4.17	6.65	2.48	1.79	−2.38
30	5.21	10.08	4.87	3.91	−1.30
42	7.44	11.16	3.72	5.95	−1.49
30° C.					
11	3.29	4.82	1.53	1.10	−2.19
20	7.27	12.02	4.75	5.89	−1.38
30	8.45	14.88	6.43	12.50	4.05
42	10.42	17.86	7.44	17.36	6.94
38.5° C.					
11	2.50	4.73	2.23	1.72	−0.78
20	6.42	9.67	3.25	11.57	5.15
30	10.08	13.89	3.81	16.89	6.81
42	11.57	15.24	3.67	19.53	7.96

These experiments are of too limited a nature to justify broad conclusions but certainly indicate that there is little justification for the statement that hot weather is unfavorable for the use of cyanamid while cool weather favors the desirable transformations. Just the reverse was the case in the experiment here reported and the indications are that low temperatures slow up the desirable changes and favor the formation of dicyanodiamid and possibly guanylurea.

NITRIFICATION OF CYANAMID AND UREA IN PARTIALLY STERILIZED SOILS

In order to determine the effect of partial sterilization of a soil upon nitrification, varying percentages of phenol were added to 100 gm. samples of soil containing 10 mgm. of nitrogen as cyanamid or urea. The percentages of phenol

are based upon the dry weight of the soil. The analyses, which were made by the colorimetric method, are given in Table VIII and figure 6.

The presence of phenol even in the smallest amounts used decreased nitrification in practically every instance and at percentages above 0.1 per cent prevented nitrification altogether. There was a very slight stimulation of nitrification at 0.01 per cent at the end of two weeks but not later. It is quite evident that phenol is very injurious to the nitrifying organisms, probably to an even greater extent than for other groups of bacteria. A partial sterilization of the soil could not, therefore, prove beneficial to nitrification if the nitrifiers were the first organisms to be killed. In Table VIII it will be observed that in most cases slightly more nitrates are present in the soils containing 1 and 2 per cent of phenol than with 0.2 to 0.4 per cent. This is probably because the larger per-

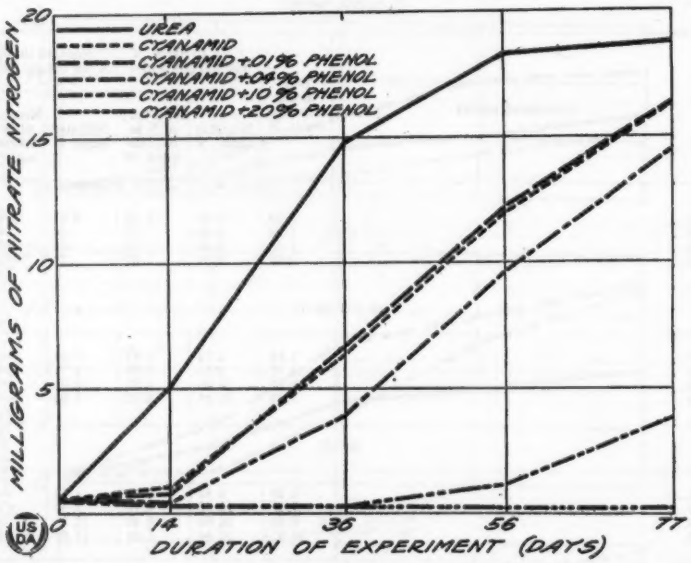

FIG. 6.—Diagram showing the effect of phenol on nitrification

centages of phenol completely sterilized the soil while with the smaller applications some groups of organisms survived and used up the nitrates present. With the highest percentages of phenol the nitrate content of the soil remained at near that of the dry soil prior to incubation. With the lower percentages the phenol practically stopped all nitrification for the first two weeks but there was a partial recovery later.

PART II. CHANGES WHICH DICYANODIAMID, GUANYLUREA, GUANIDIN, AND BIGUANID NITROGEN UNDERGO OR PRODUCE IN SOIL

COMPARATIVE RATES OF NITRIFICATION OF DICYANODIAMID, GUANYLUREA SULPHATE, AND AMMONIUM SULPHATE

This experiment was made for the purpose of determining if dicyanodiamid and guanylurea sulphate nitrify in the soil provided a considerable period of time is allowed for the process to take place. For comparison ammonium sulphate

was included. The results, which were obtained by the reduction method, are given in Table IX and figure 7.

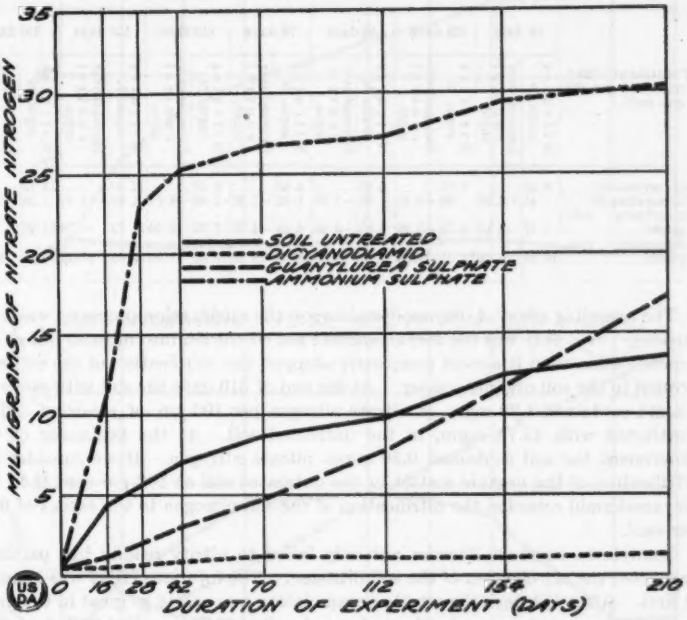

FIG. 7.—Diagram showing the nitrates present in soils receiving dicyanodiamid, guanylurea sulphate, and ammonium sulphate

TABLE VIII.—*Nitrate formation from cyanamid in soils containing various percentages of phenol* [a]

Fertilizer	Phenol	Nitrates mgm. N	Recovery of N as nitrates mgm. N	Nitrates mgm. N	Recovery of N as nitrates mgm. N
		14 days		36 days	
	Per cent				
Control	0	3.13	----------	7.81	----------
Urea	0	5.00	1.87	14.88	7.07
Cyanamid	0	.81	−2.32	6.79	−1.02
Do	0.01	1.00	−2.13	6.38	−1.43
Do	.04	.40	−2.73	3.97	−3.84
Do	.10	.27	−2.86	.32	−7.49
Do	.20	.50	−2.63	.25	−7.56
Do	.40	.69	−2.44	.27	−7.54
Do	1.00	.30	−2.83	.50	−7.31
Do	2.00	.45	−2.68	.74	−7.07
		56 days		77 days	
Control	0	8.45	----------	10.78	----------
Urea	0	18.38	9.93	18.94	8.16
Cyanamid	0	12.50	4.05	16.45	5.67
Do	.01	12.02	3.57	16.45	5.67
Do	.04	9.62	1.17	14.53	3.75
Do	.10	1.18	−7.27	3.83	−6.95
Do	.20	.21	−8.24	.25	−10.53
Do	.40	.34	−8.11	.28	−10.50
Do	1.00	.58	−7.87	.56	−10.22
Do	2.00	.78	−7.67	.50	−10.28

[a] Cyanamid and urea were used at the rate of 10 mgm. N per 100 gm. soil.

TABLE IX.—*Comparison of the rates of nitrification of dicyanodiamid, guanylurea sulphate, and ammonium sulphate*

Treatment (20.9 mgm. N per 100 gm. soil)	16 days		28 days		42 days		70 days		112 days		154 days		210 days	
	Nitrates mgm. N	Increase over control mgm.	Nitrates mgm. N	Increase over control mgm.	Nitrates mgm. N	Increase over control mgm.	Nitrates mgm. N	Increase over control mgm.	Nitrates mgm. N	Increase over control mgm.	Nitrates mgm. N	Increase over control mgm.	Nitrates mgm. N	Increase over control mgm.
Soil, untreated	4.31		5.51		6.91		8.65		9.83		12.50		13.75	
Dicyanodiamid	.45	−3.86	.66	−4.85	.87	−6.04	1.22	−7.43	1.08	−8.75	1.08	−11.42	1.29	−12.46
Guanylurea sulphate	1.17	−3.14	2.12	−3.39	3.03	−3.88	4.65	−4.00	7.73	−2.10	11.72	−.78	17.40	3.65
Ammonium sulphate	10.64	6.33	23.01	17.50	25.26	18.35	26.88	18.23	27.39	17.56	29.65	17.15	30.27	16.52

The retarding effect of dicyanodiamid upon the nitrification processes was very striking. Not only was the dicyanodiamid not converted into nitrates but at the concentration used it almost completely stopped the nitrification of the nitrogen present in the soil organic matter. At the end of 210 days the soil with dicyanodiamid contained 1.29 mgm. of nitrate nitrogen per 100 gm. of air-dried soil as contrasted with 13.75 mgm. in the untreated soil. At the beginning of the experiment the soil contained 0.56 mgm. nitrate nitrogen. If we consider the nitrification of the organic matter in the untreated soil as 100 per cent then the dicyanodiamid retarded the nitrification of the soil nitrogen to the extent of 94.5 per cent.

Guanylurea sulphate likewise not only failed to nitrify readily but partially prevented the nitrification of the soil nitrogen. The injurious effect was greatest at first. After 154 days the nitrate accumulation was nearly as great in the presence of the guanylurea as in the untreated soil. At the end of 210 days this material showed a nitrification of 17.5 per cent. These results would indicate that eventually guanylurea sulphate is transformed in the soil to nitrate nitrogen but the process is so slow that the material can not be considered as a fertilizer. In considering the injurious effects on nitrification noted in these experiments it should be borne in mind that the quantities of guanylurea required to produce the injury were far in excess of any ever likely to be found under field conditions.

THE RATE OF DECOMPOSITION OF VARYING QUANTITIES OF DICYANODIAMID

From the work reported above it seemed that dicyanodiamid was not nitrified in the soil even after a considerable period of time. In order to obtain further data on this point experiments were made using smaller quantities of the material and both ammonia and nitrate determinations were made at intervals. In analyzing the samples 25 gm. were taken for each ammonia determination while the remainder of the sample was extracted with 500 cc. of water, filtered and the dicyanodiamid nitrogen determined in 50 cc. aliquots, nitrates being determined by the reduction method in 100 cc. aliquots. The method for dicyanodiamid was tested prior to starting the experiments by analyzing several soil samples containing different known quantities of dicyanodiamid. The average recovery was 98.12 per cent with extreme limits of 95.93 and 99.34 per cent. The data, given in Table X, and shown diagrammatically in figure 8, represent the averages of duplicate determinations in the case of ammonia and nitrate nitrogen and the averages of triplicates for dicyanodiamid. The reduction method was used for the nitrate determinations.

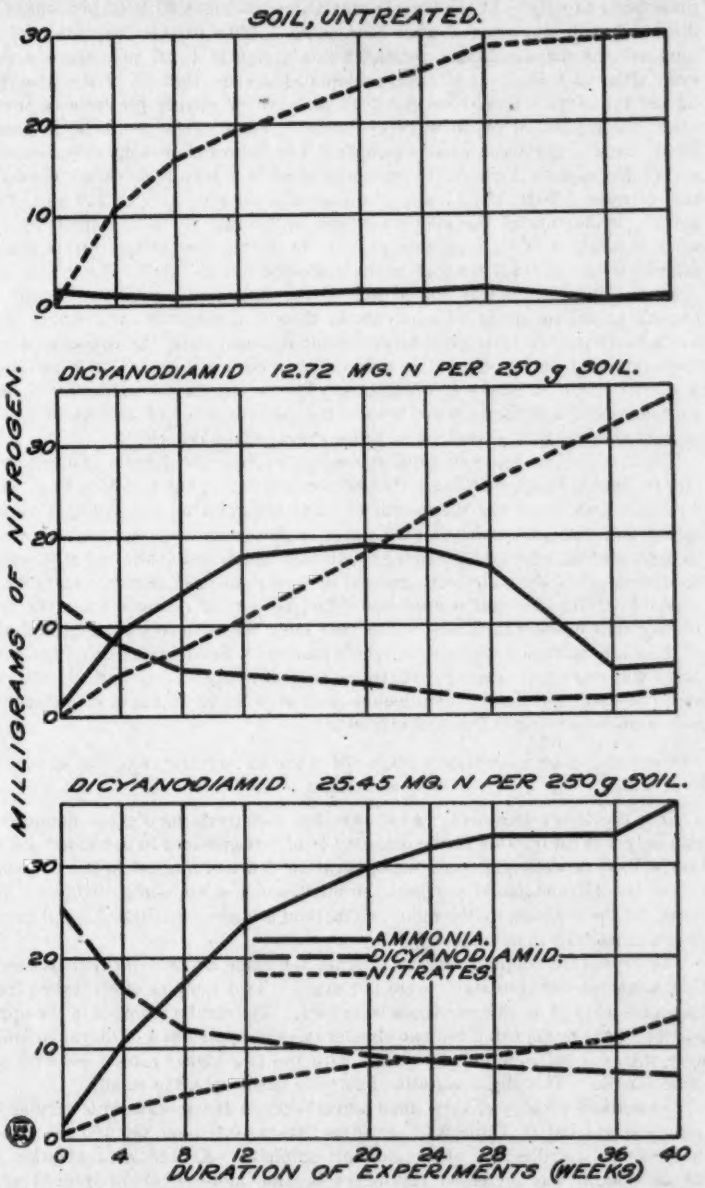

FIG. 8.—Diagram showing the rate of formation of ammonia and nitrates in soils receiving dicyandiamid

The disappearance of dicyanodiamid from the soil, as shown in Table X, takes place fairly rapidly. At the two concentrations used only 61 to 65 per cent of the dicyanodiamid was present after four weeks. With further incubation of the mixtures the dicyanodiamid decreased slowly, but 18 to 27 per cent remained even after 40 weeks. It is quite probable, however, that all of the analytic a figures for dicyanodiamid are too high since silver nitrate precipitates several other decomposition products of cyanamid. These other products, if present at all, were doubtless in small amounts. The figures show that dicyanodiamid slowly decomposes in the soil to ammonia which is in turn more slowly converted into nitrates. With 12.72 mgm. of dicyanodiamid nitrogen per 250 gm. of soil actual nitrification of the added nitrogen apparently did not commence until after 36 weeks. With twice this quantity of dicyanodiamid the nitrate present after 40 weeks was still less than in the untreated soil.

Dicyanodiamid inhibits the action of the nitrifying organisms of the soil causing an accumulation of ammonia in the soil during the early stages of its decomposition. Normal nitrification is not resumed until the concentration of undecomposed dicyanodiamid is reduced to such a point that it is no longer markedly toxic to the soil organisms. The ammonia accumulation in soils containing dicyanodiamid is due both to the decomposition of the organic matter present and also to the breaking up of the dicyanodiamid itself.

The recovery of nitrogen from the soils receiving the largest application of dicyanodiamid was never higher than 85 per cent during the first 36 weeks. This indicates that either the dicyanodiamid was absorbed by the soil to a certain extent and was not extracted with water or else some of it was converted into compounds that were not determined. In this experiment at the end of 40 weeks, apparently all of the added nitrogen was present as nitrate, ammonia and dicyanodiamid. With the smaller application the recovery of nitrogen was more than 100 per cent in some instances. This may have been due to a slight stimulation of the ammonification of the soil organic matter by the dicyanodiamid but more likely was experimental error. Extreme accuracy can not be expected with the methods used, especially when dealing with such small amounts of nitrogen in soils containing various forms of nitrogen.

THE EFFECT OF DICYANODIAMID UPON THE RATE OF NITRIFICATION OF AMMONIUM SULPHATE

Since previous experiments had shown that soils receiving dicyanodiamid contain only a small fraction of the quantity of nitrates present in untreated soil for several days or weeks following the application, it was of interest to know to what extent the dicyanodiamid prevents the nitrification of ammonia nitrogen. The results of the analyses by the reduction method are given in Table XI and shown diagrammatically in figure 9.

The procedure adopted was, in general, the same as used previously except that ammonia determinations were not made. Two samples of soil taken from the same field but at different times were used. The results reported in the upper portion of the table, using the two smaller rates of application of dicyanodiamid, were with one soil sample while those with the two higher rates were with the other sample. This slight variation had little effect upon the results.

The marked toxicity of very small percentages of dicyanodiamid is very strikingly brought out in Table XI. Even as little as 0.1 mgm. per 100 gm. of soil delayed the nitrification of ammonium sulphate. As the concentration of dicyanodiamid was increased this effect became more pronounced until at a concentration of 10.5 mgm. of dicyanodiamid per 100 gm. of soil absolutely no nitrification of ammonia nitrogen occurred in 210 days and at the end of 280 days a conversion of only 26.12 per cent of the added ammonia nitrogen had taken place

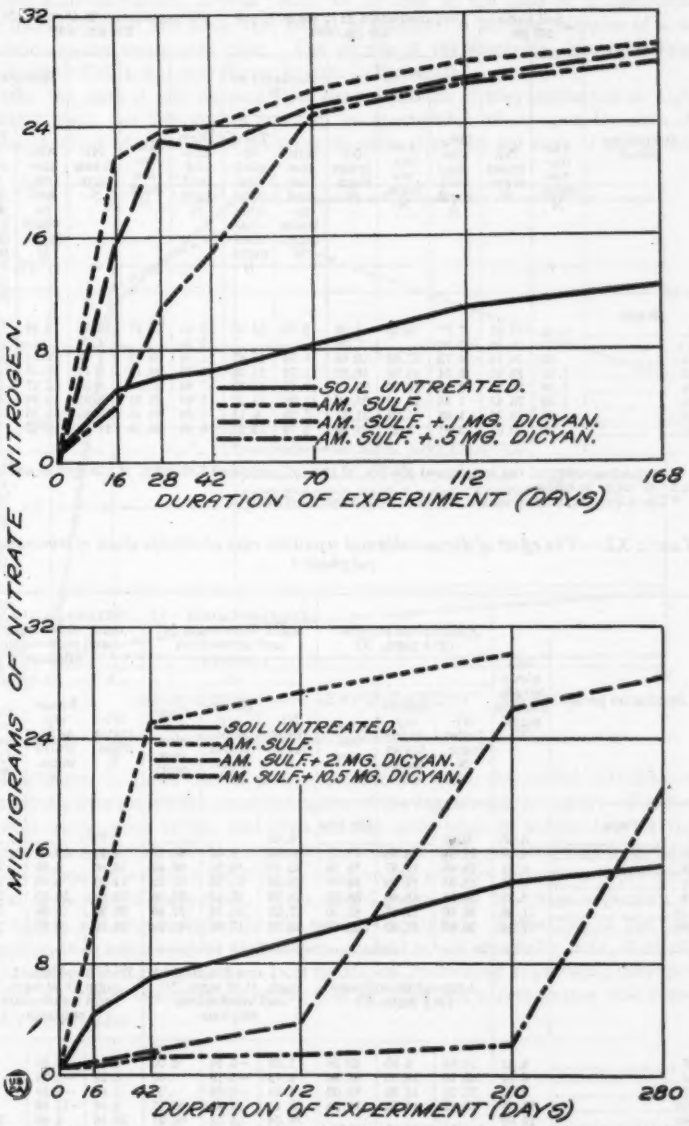

FIG. 9.—Diagram showing the effect of dicyanodiamid upon the nitrification of ammonium sulphate

TABLE X.—*Ammonification and nitrification of varying quantities of dicyanodiamid*[a]

Incubation period	Soil alone 250 gm		Dicyanodiamid 12.72 mgm. N per 250 gm. soil					Dicyanodiamid 25.45 mgm. N per 250 gm. soil				
						Recovery of N					Recovery of N	
	Ammonia mgm. N	Nitrates mgm. N	Dicyanodiamid mgm. N	Ammonia mgm. N	Nitrates mgm. N	Ammonia and nitrates mgm. N	Total, including dicyanodiamid mgm. N	Dicyanodiamid mgm. N	Ammonia mgm. N	Nitrates mgm. N.	Ammonia and nitrates mgm. N	Total, including dicyanodiamid mgm. N
Weeks												
4	0.43	10.61	7.77	9.38	4.56	2.90	10.67	16.65	10.47	3.25	2.68	19.33
8[b]		16.16	5.20		7.20			12.18		4.73		
12	.83	19.14	4.73	17.63	10.48	8.14	12.87	11.04	23.72	5.99	9.74	20.78
20	1.35	23.91	3.54	18.96	18.02	11.72	15.26	9.30	29.18	8.11	12.03	21.33
24	1.30	25.91	2.40	18.26	22.14	13.19	15.59	7.58	31.80	8.38	12.97	20.55
28	1.52	28.43	1.36	16.41	25.50	11.96	13.32	7.80	33.21	8.92	12.18	19.98
36	.00	29.26	1.86	5.04	31.51	7.29	9.15	7.24	33.16	10.45	14.35	21.59
40	.39	30.07	2.51	5.22	34.98	9.74	12.25	6.83	36.58	12.80	18.92	25.75

[a] At the beginning of the experiment 250 gm. of the soil contained 1.88 mgm. of nitrogen as ammonia and 1.63 mgm. as nitrate.

[b] The ammonia determinations were lost at this period of analysis.

TABLE XI.—*The effect of dicyanodiamid upon the rate of nitrification of ammonium sulphate*[a]

Incubation period	Soil alone 100 gm. nitrates mgm. N	Ammonium sulphate (20.9 mgm. N)			Dicyanodiamid 0.1 mgm. (0.066 mgm. N) and ammonium sulphate			Dicyanodiamid 0.5 mgm. (0.331 mgm. N) and ammonium sulphate		
		Nitrates mgm. N	Recovery as nitrates mgm. N	Conversion	Nitrates mgm. N	Recovery as nitrates mgm. N	Conversion (per cent)	Nitrates mgm. N	Recovery as nitrates mgm. N	Conversion (per cent)
Days				*Per cent*						
0	0.65	0.65			0.65			0.65		
16	4.98	21.64	16.66	79.71	14.63	9.65	46.17	4.18	−0.80	0.00
28	6.01	23.48	17.47	83.59	22.82	16.81	80.43	10.66	4.65	22.25
42	6.45	24.05	17.60	84.21	22.38	15.93	76.22	14.53	8.08	38.60
70	8.24	26.64	18.40	88.04	25.39	17.15	82.06	24.89	16.65	79.66
112	11.03	28.50	17.47	83.59	27.22	16.19	77.46	26.99	15.96	76.36
168	12.34	29.83	17.49	83.68	29.35	17.01	81.39	28.69	16.35	78.23
		Ammonium sulphate (20.9 mgm. N)			Dicyanodiamid 2 mgm. (1.33 mgm. N) and ammonium sulphate			Dicyanodiamid 10.5 mgm. (6.96 mgm. N) and ammonium sulphate		
16	4.31	10.64	6.33	30.28	1.05	−3.26	0.00	0.94	−3.37	0.00
42	6.91	25.26	18.35	87.80	1.73	−5.18	.00	1.25	−5.66	.00
112	9.83	27.39	17.56	84.02	3.94	−5.89	.00	1.44	−8.39	.00
210	13.75	30.27	16.52	79.04	26.41	12.66	60.57	2.36	−11.39	.00
280	15.00				28.58	13.58	64.97	20.46	5.46	26.12

[a] Ammonium sulphate was used in all cases at the rate of 20.9 mgm. N per 100 gm. of soil.

THE EFFECT OF DICYANODIAMID UPON THE RATE OF AMMONIFICATION OF UREA

These experiments were planned to determine if dicyanodiamid prevents or retards ammonification, as was found to be true in the case of nitrification. The dicyanodiamid and urea were added in solution to 250 gm. samples of soil. No calcium carbonate was used. The results of the ammonia determinations are given in Table XII and shown in part in figure 10.

From the data it will be seen that concentrations of dicyanodiamid as high as 315.2 mgm. per 250 gm. of soil had no appreciable effect upon the rate of ammonification of urea. The recovery of more than 100 per cent of the added

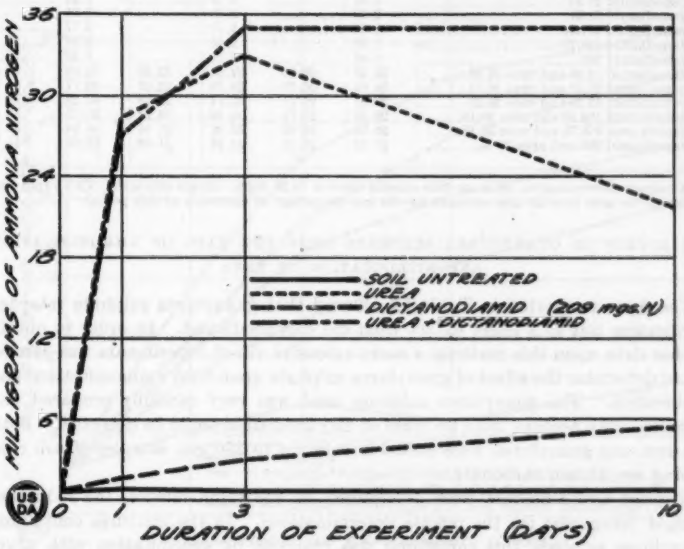

FIG. 10.—Diagram showing the effect of dicyanodiamid upon the ammonification of urea

urea nitrogen in most of the analyses indicates that the added nitrogenous compounds accelerated the ammonification of the organic soil nitrogen. Another possible explanation is the fact that soluble salts tend to accumulate at the surface of a soil layer due to evaporation, and it is quite possible that in obtaining the 25 gm. of soil from the 250 gm. samples the mixture was not as thoroughly mixed as it should have been. This would mean that too large a portion of the surface soil was included in the samples analyzed. Regardless of the discrepancy, it is quite evident that dicyanodiamid is not especially toxic, if at all, to the ammonifying organisms. In a fertile soil containing appreciable dicyanodiamid we would therefore expect to find an ammonia accumulation and practically no nitrates.

TABLE XII.—*The effect of dicyanodiamid upon the ammonification of urea*

Treatment (mgm. N per 250 gm. soil)	1 day		3 days		10 days	
	Ammonia mgm. N	Urea as ammonia mgm. N	Ammonia mgm. N	Urea as ammonia mgm. N	Ammonia mgm. N	Urea as ammonia mgm. N
Soil alone	0.83	----------	0.65	----------	0.54	----------
Urea 26.15	28.43	----------	32.98	32.33	22.37	[a] 21.83
Dicyanodiamid 13.06	1.40	----------	2.10	----------	2.85	----------
Dicyanodiamid 26.12	1.88	----------	2.63	----------	3.33	----------
Dicyanodiamid 52.24	2.10	----------	3.95	----------	3.81	----------
Dicyanodiamid 104.50	1.88	----------	3.73	----------	3.77	----------
Dicyanodiamid 156.75	1.66	----------	2.45	----------	3.38	----------
Dicyanodiamid 209	1.62	----------	2.98	----------	3.33	----------
Dicyanodiamid 13.06 and urea 26.15	28.25	26.85	34.65	32.55	38.07	35.22
Dicyanodiamid 26.12 and urea 26.15	28.64	26.76	34.70	32.07	38.77	35.44
Dicyanodiamid 52.24 and urea 26.15	27.81	25.71	36.14	32.19	37.28	33.47
Dicyanodiamid 104.50 and urea 26.15	27.59	25.71	36.49	32.76	39.17	35.40
Dicyanodiamid 156.75 and urea 26.15	26.06	24.40	33.91	31.46	34.34	30.96
Dicyanodiamid 209 and urea 26.15	27.15	25.53	34.96	31.98	35.09	31.76

[a] A nitrate determination made on this sample showed 14.26 mgm. nitrate nitrogen This rapid conversion of the urea into nitrates accounts for the low percentage of ammonia at this period.

THE EFFECT OF GUANYLUREA SULPHATE UPON THE RATE OF AMMONIFICATION AND NITRIFICATION OF UREA

The figures reported in Table IX showed that guanylurea sulphate retarded nitrification but to a lesser extent than did dicyanodiamid. In order to obtain further data upon this material a more extensive set of experiments was carried out to determine the effect of guanylurea sulphate upon both ammonification and nitrification. The guanylurea sulphate used was very carefully prepared and extracted with acetone until no trace of dicyanodiamid could be detected. Both the urea and guanylurea were added in solution to 250 gm. samples of soil containing no calcium carbonate.

Nitrates and ammonia were determined on the same sample, the reduction method being used for the nitrate determinations. In the mixtures containing guanylurea sulphate this compound was removed by precipitation with silver sulphate and potassium hydroxid before analyzing for nitrates. The data are given in Table XIII. Figure 11 was based upon these results.

It will be seen that guanylurea sulphate, in the concentrations used in the above experiments, had very little if any effect upon the rate of conversion of urea to ammonia in the soil but it did markedly slow down the nitrification of this material. The higher the concentration of guanylurea sulphate the greater was the inhibiting effect but it was not nearly as great as in the case of dicyanodiamid. Where guanylurea sulphate was applied singly there was no ammonia accumulation as in the case of dicyanodiamid (Table X) but the material did slowly break up into ammonia. The small quantity formed was converted into nitrates almost immediately. Comparing the results of Table XIII with those of Table X it will be seen that while dicyanodiamid is converted into ammonia fairly rapidly a very small amount of the material will prevent the oxidation of the ammonia to nitrates. On the other hand, guanylurea ammonifies only very slowly but it is much less toxic to the nitrifying organisms. From these results we would conclude that both of the compounds, particularly dicyanodiamid, are undesirable in soils. However, it should be emphasized that since such exceedingly large percentages of guanylurea sulphate are required to appreciably slow up nitrification there is little probability that injury would be observed under practical conditions.

NITRIFICATION OF GUANIDIN NITRATE, NITRO GUANIDIN, BIGUANID NITRATE AND UREA

In continuation of the study of the transformation products of cyanamid a series was started using guanidin nitrate, nitro guanidin and biguanid nitrate with urea for comparison. The undesirability of using nitrate salts of the organic radicals was appreciated but at the time of starting the experiments only these salts were available. Adequate controls were arranged where the soil received

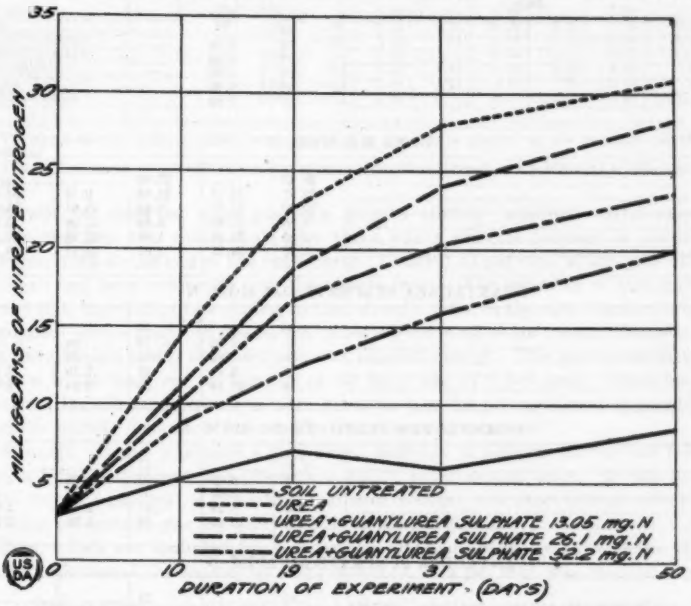

FIG. 11.—Diagram showing the effect of guanylurea sulphate upon the rate of nitrification of urea

sodium nitrate equivalent to the nitrate nitrogen in the compounds being studied. The rate of application for all of the salts was 10 mgm. nitrogen other then nitrate per 100 gm. soil. The colorimetric method was used for nitrate determinations. The data which are the averages of duplicate determinations are given in Table XIV.

TABLE XIII.—*Ammonification and nitrification of urea in the presence of guanylurea sulphate*

Time	Ammonia mgm. N	Nitrates mgm. N	Recovery of Nitrogen[a] As ammonia mgm. N	Recovery of Nitrogen[a] As nitrates mgm. N	Recovery of Nitrogen[a] Total mgm. N
	SOIL ALONE (250 GM.)				
Days					
0	1.31	3.07			
1	.83				
10	.54	5.08			
19	.17	7.04			
31	.18	5.92			
50	.00	8.50			
100	.31	9.66			
	UREA 26.15 MGM. N				
1	28.43		27.60		
10	22.37	14.26	21.83	9.18	31.01
19	10.00	22.65	9.83	15.61	25.44
31	3.86	27.88	3.68	21.96	25.64
50	1.88	30.88	1.88	22.38	24.26
100	1.93	34.37	1.62	24.71	26.33
	GUANYLUREA SULPHATE 13.05 MGM. N				
1	1.53		.70		
10	.41	5.92	−.13	.84	.71
19	.20	7.83	.03	.79	.82
31	.45	8.71	.27	2.79	3.06
50	.54	10.84	.54	2.34	2.88
	GUANYLUREA SULPHATE 26.1 MGM. N				
1	1.40		.57		
10	.76	6.36	.22	1.28	1.50
19	.18	7.64	.01	.60	.61
31	.45	8.29	.27	2.37	2.64
50	.48	10.29	.48	1.79	2.27
	GUANYLUREA SULPHATE 52.2 MGM. N				
1	1.62		.79		
10	3.16	6.09	2.62	1.01	3.63
19	1.66	8.71	1.49	1.67	3.16
31	1.75	8.21	1.57	2.29	3.86
50	.65	9.02	.65	.52	1.17
	GUANYLUREA SULPHATE 13.05 MGM. N—UREA 26.15 MGM.				
1	26.49		24.96		
10	28.69	11.06	28.28	5.14	33.42
19	22.15	18.60	21.95	10.77	32.72
31	19.43	24.04	18.98	15.33	34.31
50	12.98	28.32	12.44	17.48	29.92
	GUANYLUREA SULPHATE 26.1 MGM. N—UREA 26.15 MGM. N				
1	26.75		25.35		
10	30.44	10.21	29.68	3.85	33.53
19	27.68	16.75	27.50	9.11	36.61
31	25.57	20.17	25.12	11.88	37.00
50	20.61	23.54	20.13	13.25	33.38
	GUANYLUREA SULPHATE 52.2 MGM. N—UREA 26.15 MGM. N				
1	25.70		24.08		
10	31.20	8.71	28.04	2.62	30.66
19	27.88	12.32	26.22	3.61	29.83
31	30.13	15.93	28.38	7.72	36.10
50	27.58	20.07	26.93	11.05	37.98

[a] The figures for the recovery of nitrogen apply to urea only, where guanylurea sulphate and urea were used together

TABLE XIV.—*Comparison of the rates of nitrification of guanidin nitrate, nitro guanidin, biguanid nitrate and urea*

Treatment	Mgm. N added other than nitrates	14 days		29 days		50 days		71 days	
		Nitrates mgm. N	Increase over control mgm.	Nitrates mgm. N	Increase over control mgm.	Nitrates mgm. N	In. crease over control mgm.	Nitrates mgm. N	Increase over control mgm.
Soil alone	0	3.85		5.00		6.94		7.91	
Urea	10	4.93	1.08	10.78	5.78	14.20	7.26	17.36	9.45
Guanidin nitrate	10	5.65	−.60	8.33	.52	11.61	1.62	12.50	3.31
Sodium nitrate[a]	0	6.25		7.81		9.99		9.19	
Nitro guanidin	10	4.71	.86	6.10	1.10	8.68	1.74	8.80	.89
Biguanid nitrate	10	4.98	−.32	7.14	−.21	9.19	.97	8.91	.90
Sodium nitrate [b]	0	5.30		7.35		8.22		8.01	

[a] The application of sodium nitrate was equivalent to the nitrate present in the guanidin nitrate treatment.

[b] The application of sodium nitrate was equivalent to the nitrate present in the biguanid nitrate treatment.

It will be observed that guanidin nitrate slightly inhibited nitrification during the first two weeks but later there was a gradual increase in nitrate nitrogen until at the end of the experiment (71 days) 33 per cent of the guanidin nitrogen had been converted into the nitrate form. The behavior of this compound was, therefore, quite similar to that already noted in the case of guanylurea sulphate. Nitro guanidin did not have an injurious effect on nitrate formation but nevertheless it did not nitrify to any marked extent. The maximum nitrification which occurred at the end of 50 days was 17.4 per cent. Since one-fourth of the nitrogen present in nitro guanidin is in the nitrite form it is possible that the nitrate formation was wholly from the nitrite radical.

Biguanid nitrate produced a very slight decrease in nitrates during the first month but the decrease was practically within experimental error. At the end of 50 days 9.7 per cent of the added biguanid nitrogen was recovered as nitrates and this percentage was not increased by longer incubation.

Urea, which was included for comparison, nitrified gradually until at the end of the experiment the maximum nitrification of 94.5 per cent was reached.

NITRIFICATION OF GUANIDIN CARBONATE AND AMMONIUM SULPHATE

Subsequent to starting the experiment discussed above a sample of guanidin carbonate was obtained and a new series of experiments started similar to the first using this material in comparison with ammonium sulphate. Three rates of application were used namely, 5, 10 and 20 mgm. N per 100 gm. soil. The figures given in Table XV are the average of duplicate determinations obtained by the colorimetric method. These results are also shown in figure 12.

TABLE XV.—*Comparison of the rates of nitrification of guanidin carbonate and ammonium sulphate*

Treatment	Mgm. N added	19 days		35 days		56 days		75 days	
		Nitrates mgm. N	Increase over control mgm.	Nitrates mgm. N	Increase over control mgm.	Nitrates mgm. N	Increase over control mgm.	Nitrates mgm. N	Increase over control mgm.
Soil alone	0	6.86		7.66		8.99		8.54	
Ammonium sulphate	5	10.21	3.35	11.58	3.92	14.48	5.49	13.72	5.18
Do	10	10.00	3.14	16.95	9.29	18.95	9.96	18.95	10.41
Do	20	9.44	2.58	26.39	18.73	28.18	19.19	27.79	19.25
Guanidin carbonate	5	4.68	−2.18	10.22	2.56	12.57	3.58	12.71	4.17
Do	10	2.07	−4.79	9.31	1.65	15.43	6.44	17.66	9.12
Do	20	1.38	−5.48	1.71	−5.95	9.74	.75	18.28	9.74

It will be observed that guanidin carbonate very markedly inhibited nitrification during the first three to five weeks, depending upon the rate of application. Later the nitrification was quite rapid and nearly complete. At the last period of analysis the percentage nitrification was 83.4, 91.2, and 48.7 for the 5, 10, and 20 mgm. of nitrogen added. Undoubtedly, if the experiment had been continued

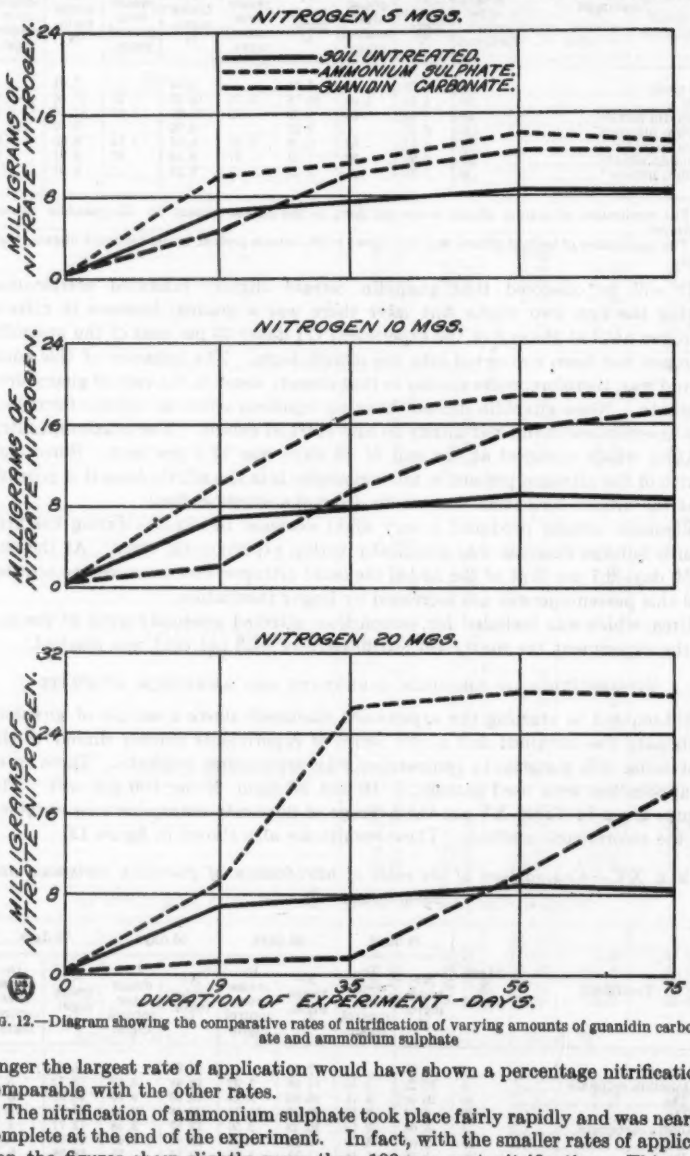

FIG. 12.—Diagram showing the comparative rates of nitrification of varying amounts of guanidin carbonate and ammonium sulphate

longer the largest rate of application would have shown a percentage nitrification comparable with the other rates.

The nitrification of ammonium sulphate took place fairly rapidly and was nearly complete at the end of the experiment. In fact, with the smaller rates of application the figures show slightly more than 100 per cent nitrification. This was probably due to a slight stimulation of nitrification of the soil organic matter.

SUMMARY

The study of the changes which cyanamid nitrogen and its transformation products undergo in the soil emphasized the following points:

1. Cyanamid was rapidly converted into its various decomposition products, chiefly urea and ammonia. Tests for cyanamid showed none present after 5 to 10 days depending upon the rate of application. Urea was likewise present for only very short periods, being broken up into ammonia so rapidly that no accumulation occurred. Other decomposition products of cyanamid which were precipitated by silver nitrate were present in soils receiving cyanamid for a considerable period after applying. No attempt was made to isolate these compounds but they probably consisted largely of dicyanodiamid and possibly guanylurea.

2. The nitrification of cyanamid usually took place more slowly than that of urea or ammonium sulphate and the larger the application the slower the process. After the initial retarding period of two to four weeks nitrification proceeded at a more nearly normal rate. Some of the transformation products of cyanamid are toxic to the nitrifying bacteria and where present in appreciable amounts indirectly cause an ammonia accumulation.

3. Cyanamid which had been hydrated and oiled nitrified at practically the same rate as did the untreated material. The slight difference in favor of the latter is thought to have been due to the presence of a trace of dicyanodiamid in the hyrdated material and not to the presence of the oil.

4. The addition of calcium carbonate to soil receiving cyanamid retarded nitrification, contrary to results obtained with urea and ammonium sulphate. This peculiar behavior was probably due to an increase in dicyanodiamid formation in the presence of the calcium carbonate.

5. The rate of nitrification of cyanamid at different moisture contents was the highest at one-fourth of saturation (10 per cent) while for urea the rate was higher with both one-half and three-fourths than with one-fourth. The maximum nitrate accumulation for both fertilizers was with 30 per cent moisture but in the case of cyanamid the nitrates present after two months was very nearly the same with either 10, 21 or 30 per cent. With 40 per cent moisture there was little if any nitrate formation.

6. Nitrate formation from cyanamid was more rapid and complete at 38.5° C. than at lower temperatures. At room temperature no nitrification had taken place in one experiment after 42 days. At 30° C. the results were intermediate. Under similar conditions urea nitrified at all temperatures, the rate rapidly increasing up to 30° C. but decreasing at 38.5° C.

7. The partial sterilization of soil with phenol practically stopped all nitrification of cyanamid.

8. Dicyanodiamid when added to soil slowly disappeared, more than one-half being decomposed during a period of two months. The nitrogen accumulated in the soil as ammonia which was not readily nitrified. With 12.72 mgm. of nitrogen as dicyanodiamid per 250 gm. of soil 36 weeks were required for any of the added nitrogen to nitrify while with larger applications no nitrification took place in 40 weeks. In all cases where dicyanodiamid was applied the nitrate formation from the soil organic matter was markedly retarded.

9. The nitrification of ammonium sulphate in the presence of dicyanodiamid was prevented for a period of 210 days where the dicyanodiamid was used at the rate of 10.5 mgm. per 100 gm. soil. Even as little as 0.1 mgm. per 100 gm. of soil greatly delayed nitrification.

10. The rate of ammonification of urea was not appreciably affected by concentrations of dicyanodiamid as high as 315.2 mgm. per 250 gm. soil.

11. Guanylurea sulphate decomposed to ammonia very slowly and the ammonia so formed did not accumulate but was nitrified. Where urea was used with guanylurea sulphate the latter did not affect ammonification but did inhibit nitrification for some weeks. The injurious effect was not nearly as great as in the case of dicyanodiamid and would probably not be observed at all under field conditions.

12. Salts of guanidin, including guanidin nitrate and guanidin carbonate depressed nitrification for several weeks, the period depending upon the rate of application. Thereafter nitrates formed quite rapidly and after 75 days guanidin carbonate had nitrified to the extent of 83, 91 and 49 per cent for the three rates of application used. Nitro guanidin showed a maximum nitrification of 17 per cent after 50 days.

13. Biguanid nitrate acted practically like an inert material. A 3 per cent depression in nitrates at first was followed by a 9 per cent nitrification after 50 days. These slight differences are nearly within experimental error.

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GROSSULARIA ECHINELLA, A SPINY-FRUITED GOOSEBERRY FROM FLORIDA[1]

By FREDERICK V. COVILLE

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While in Tallahassee, Fla., early this spring, the writer was invited by Dr. Roland M. Harper, of the State geological survey, and Dr. H. Kurz, of the State College for Women, to join them in a visit to Lake Miccosukee where a few weeks before they had found a wild gooseberry, at that time not yet in flower. The plant promised to be of great interest, for although the name "gooseberry" is often misapplied in the southeastern United States to the fruit of the various species of deerberry, or Polycodium, no true gooseberry had ever been reported from Florida.

On March 2, 1924, we made the 26-mile drive from Tallahassee to Dogwood Landing, on the east shore of the northwest arm of Lake Miccosukee, about a mile east of the main buildings of the Norias Club. A few hundred yards east of the landing, in the strip of woodland bordering the lake, we came upon the first plant, in full leaf and flower. It had been our expectation that it would prove to be the Georgia gooseberry, *Grossularia curvata*, a rare southern species, the type locality of which is on the slopes of Stone Mountain, Ga. The plant found, however, was recognized immediately as not *Grossularia curvata* but a species new to science, differing conspicuously from *curvata* in the coarse bristly gland-tipped hairs of the ovary, which develops into an exceedingly spiny fruit. This characteristic has suggested the species name *echinella*, which indicates the resemblance of the berry to a little hedgehog.

On March 27 Doctor Kurz and the writer again visited the locality. Unusually cold weather had prevailed and many of the gooseberry bushes were still in full flower. Some of the young green spiny fruits had already reached a diameter of five-eighths of an inch. On April 7 Doctor Kurz visited the place again and sent the writer fresh pollen from newly expanded flowers, and half mature fruits, the largest seven-eighths of an inch in diameter.

TECHNICAL DESCRIPTION

Grossularia echinella, sp. nov.

Plant a shrub 0.5 to 1 meter in height, often forming patches several meters in diameter, the branches spreading and recurved, sometimes rooting at the tip; stems with spines at the nodes but devoid of bristles except occasionally on vigorous basal shoots; spines single, double, or triple, stout, dark reddish brown, sometimes turning gray with age, reaching a length of 1.5 cm.; outer bark of 1-year-old branches dull white to almost buff, splitting with the growth of the twig and exposing the inner dark reddish brown bark; petioles pubescent, usually a little longer than the leafblades but sometimes exceeded by them and usually bearing near the base a few large gland-tipped and often plumose hairs;

[1] Received for publication, Apr. 1, 1924.

leafblades 1 to 2 cm. long, sometimes smaller, on vigorous shoots of the season, longer, on strong basal sprouts sometimes reaching 3 cm., orbicular in outline, truncate to rounded at the base, 3-lobed, each lobe with a few rounded teeth, sparingly pubescent or nearly glabrous on both surfaces; peduncles 5 to 12 mm. long, pubescent and usually with scattered longer gland-tipped hairs, one-flowered or occasionally two-flowered; bracts usually 2, pubescent, entire; pedicels 1 to 5 mm. long, densely pubescent and often with scattered gland-tipped hairs like those of the ovary; flower 15 to 20 mm. in length, green to greenish white; ovary densely clothed with stout bristly hairs tipped with cup-shaped glands; calyx tube green, pubescent on the outside, sparingly pubescent on the inside toward the base, cylindrical, 3 to 4 mm. long, the lobes green to greenish white, linear, pubescent on the outside, glabrous on the inside, reflexed, 4 to 6 mm. long; petals about 2 mm. long, greenish white to green, each inrolled into a truncate tube with a spreading or revolute rim at the summit; stamens extending nearly 1 cm. beyond the petals; filaments greenish white to green, usually sparingly hairy; anthers purple, about 1.5 mm. long when dry, sometimes 2.5 mm. long when fresh, usually with a line of long white hairs (or occasionally a green and gland-tipped hair) on the back between the anther sacs; styles green, a little longer than the stamens, very sparingly hairy within, as well as beyond, the calyx tube; largest berry, not yet mature, 22 mm. long, 22 mm. broad, including the prickles, the body 16 mm. in length, 12 mm. in thickness, translucent, uncolored;[2] fruit prickles very numerous, vivid green, somewhat flattened, longer toward the middle of the berry than at the ends, reaching a length of 5 mm., the larger nearly 1 mm. wide at the base, each ending in a green cup-shaped gland. (See Pl. 1.)

Type specimen No. 1,112,807, United States National Herbarium, collected March 2, 1924, a few hundred yards south of Dogwood Landing, east shore of the northwest arm of Lake Miccosukee, Jefferson County, Florida, by Frederick V. Coville.

In the somber surroundings of gray tree trunks, gray Spanish-moss (*Dendropogon usneoides*), and gray-brown leaf litter, the patches of this plant were conspicuous for the brilliant green of their new foliage. The only other shrub conspicuously green at the time the gooseberry came into bloom was red buckeye, *Aesculus pavia*, which also was in full leaf and nearly ready to flower. Although its stems are viciously spiny, the new Grossularia when in bloom is of graceful and pleasing appearance, the slender, elegant, pale green flowers hanging down singly from the broadly arching branches like aquamarines pendent from an emerald necklace.

The new species differs from its nearest relative, *Grossularia curvata*, in the white or whitish color of the 1-year-old twigs; the ovary densely clothed with stout gland-tipped hairs; the cylindrical, pubescent calyx tube 3 to 4 mm. in length and longer than broad; the green or greenish color of the calyx lobes; the inrolled tubular character of the petals; and the spiny fruit, which looks like a cocklebur, especially like the fruit of *Xanthium globosum*. In *Grossularia curvata* the 1-year-old twigs are dark reddish brown; the ovary bears sessile glands; the calyx tube is saucer-shaped, hardly more than a millimeter in length, much broader than long, and smooth or nearly so; the calyx lobes white; petals expanded and toothed; and fruit smooth. In the new species the filaments and style are only very sparingly hairy, in *curvata* densely and conspicuously hairy.

[2] Berries collected by Doctor Harper on May 10, though still hard, green, and unripe, had reached the astonishing diameter of 30 mm. (over 1⅛ inches), including the spines, and the body of the berry 22 mm. (over ¾ of an inch).

In the new species the sepals when reflexed reach to the summit or sometimes to the base of the ovary, in *curvata* far beyond its base.

On the pollination of *Grossularia echinella* only a few observations were made. Isolated plants did not set as much fruit as plants growing in groups and therefore better situated for cross-pollination. Only a single insect was seen pollinating the flowers, a carpenter bee, *Xylocopa virginica*. To suck the nectar it alighted in an inverted position on the pendent flower, grasping the stamens and style with all its legs in such a manner that the anthers and stigmas were brought into contact with the middle of the underside of its body, a very effective position for the transfer of pollen from one flower to another and from one plant to another. The bee assumed its inverted perch instantly, without hesitation and without slipping, drew the nectar rapidly, and proceeded promptly to another flower. In maintaining a firm grip on its perch it must have been greatly aided by the long hairs on the filaments of the flower, a characteristic very unusual in the genus Grossularia, and occurring in no other of the more than 40 American species except *curvata* and *nivea*. Both of these, like *echinella*, have long protruding stamens which, if devoid of hairs, would furnish only a slippery hold to a pollinating insect.

The cup-shaped glands at the tips of the coarse hairs that densely clothe the ovary were not yet yielding an exudate either in the newly opened flowers or in the older withered flowers in which the hairs had begun their elongation into prickles, a condition that suggested, at the time of the first observation of the inflorescence, that the function of the glands was related to the fruit, not to the flower. This suggestion was confirmed by the observations made on March 27 and by those made by Doctor Kurz on April 7 and later, when some of the berries were half mature. As the fruit enlarges, the glands at the ends of the prickles give off a sticky and unpalatable secretion. The species presents therefore the apparent anomaly of a sweet and succulent berry well adapted to the dispersal of its seeds by fruit-eating animals, yet barred from this means of dispersal by its spiny covering and offensive exudate. A little consideration, however, discloses the fact that these obstacles, though effective against such animals as insects, rabbits, and squirrels, would present only an interesting conundrum to such wide-awake and investigative fruit-eating animals as mocking birds, catbirds, and thrushes, whose long bills would enable them to open the berries with ease, and whose digestive limitations would insure a wide distribution of the living seeds. The structures that appear at first, therefore, as a bar against seed dispersal provide in all probability a very special and very effective means of advancing the distribution of the species.

At the present time the new species is known only from the type locality, on the north side of Lake Miccosukee, Fla. *Grossularia curvata* occurs in northern Georgia, northern Alabama, Louisiana, and eastern Texas. It may be questioned why a shrub so vigorous, so well protected against grazing animals, and so well adapted to dissemination by fruit-eating birds as *Grossularia echinella*, has such a limited geographical range, a strip of country about a mile in length and only a few rods in width. The manner of occurrence of the bushes in this area indicates that the establishment of the species here is of comparatively recent date. The plants occur in a definite center of abundance, with many individual bushes forming thickets, and farther away individual younger plants more widely separated from each other. The situation is exactly what would be expected if seeds of this species had been first introduced into this locality a few decades ago from some other and older center of distribution. The present known area of the species appears to be an advance colony, not a remnant, and it is to be expected that another and parent area will sometime be

discovered.[3] That the plants have not come from seeds scattered by birds from some foreign species cultivated in the neighborhood of Lake Miccosukee is evidenced by the fact that *Grossularia echinella* is very different from any of the species of Asia, Europe, or the mountains of northern Africa, the only parts of the world besides North America in which Grossularias are native.

The spininess of the fruit is the most remarkable characteristic of *Grossularia echinella*. Of the six other species of gooseberry native in eastern North America, *cynosbati*, *oxyacanthoides*, *hirtella*, *rotundifolia*, *missouriensis*, and *curvata*, only one, *cynosbati*, has spiny fruit, and that species is not closely related to the present species, as shown by their very different flower structure. Even in *cynosbati* the prickles of the fruit are comparatively few, in *echinella* they occur in hundreds. It is only in some of the Pacific coast species, such as *menziesii*, *hesperia*, and *hystrix*, that such dense spininess occurs, and the flower structure of all such spiny-fruited Pacific coast species shows that none of them is closely related to the new one.

Grossularia echinella is closely related to three other American gooseberries: *G. curvata*, of the southeastern United States; *missouriensis*, of the middle and upper Mississippi Valley region; and *nivea*, of the plains of eastern Washington and Oregon, western Idaho, and northern Nevada, a species which, though now stranded far from the others geographically, presents evidence of close genetic relationship with them, especially with *Grossularia curvata*. None of these species, however, has spiny fruit. But in *curvata* the ovaries are densely covered with sessile glands, and the elevation of these glands on stalks, a tendency which often appears in Grossularia and also in the related genus Ribes, provides a reasonable explanation of the evolution of the gland-tipped prickles of the new species.

In its geographic distribution *Grossularia echinella* is of special interest because it is the southernmost of our Atlantic seaboard species of this genus, growing in Florida, at an elevation of only about 200 feet above sea level, and in the principal region of production of the Satsuma orange. It may therefore be regarded as an almost subtropical representative of a north temperate genus. The cultivation of our present garden gooseberries in the latitude to which they are adapted, the Northern States, is now discouraged by forestry experts because the gooseberries, like the currants, are carriers of a blister rust that threatens the destruction of the white-pine forests. The danger is so seriously regarded that more than a million dollars has already been expended in the eradication of gooseberries and currants in the white-pine region. Should it be judged desirable that the agricultural range of the cultivated gooseberries be extended farther south than it is now possible to grow them, and that an attempt be made to establish gooseberry culture beyond the range of the white-pine forests, the new species offers a southern climatic adaptation which it may be possible to combine with the edible qualities of the garden gooseberries through hybridization. The culture of gooseberries in the southern coastal plain would carry no menace to the pine forests of that region because the hard pines are immune to the blister rust of the white pine, and, furthermore, the gooseberries in that region would not even have the disease because there are no white pines from which to contract it.

[3] The prediction of a parent area has come true sooner than was expected. Doctor Kurz visiting Lake Miccosukee again on April 27 found that about a mile farther from Dogwood Landing the new species was running rampant as the dominant shrub of the forest belt and extending beyond it into the upland along the slopes of small streams emptying into the lake.

PLATE 1

A.—Flowering branch. Natural size.

B.—Portion of a stem showing the cracked outer bark, and the stout nodal spines projecting backward. Natural size.

C.—Petal viewed from the inside of the pendent flower. Note the tubular form of the petal, brought about by the inrolling of the margins. A portion of a filament is shown beside the petal. ×4.

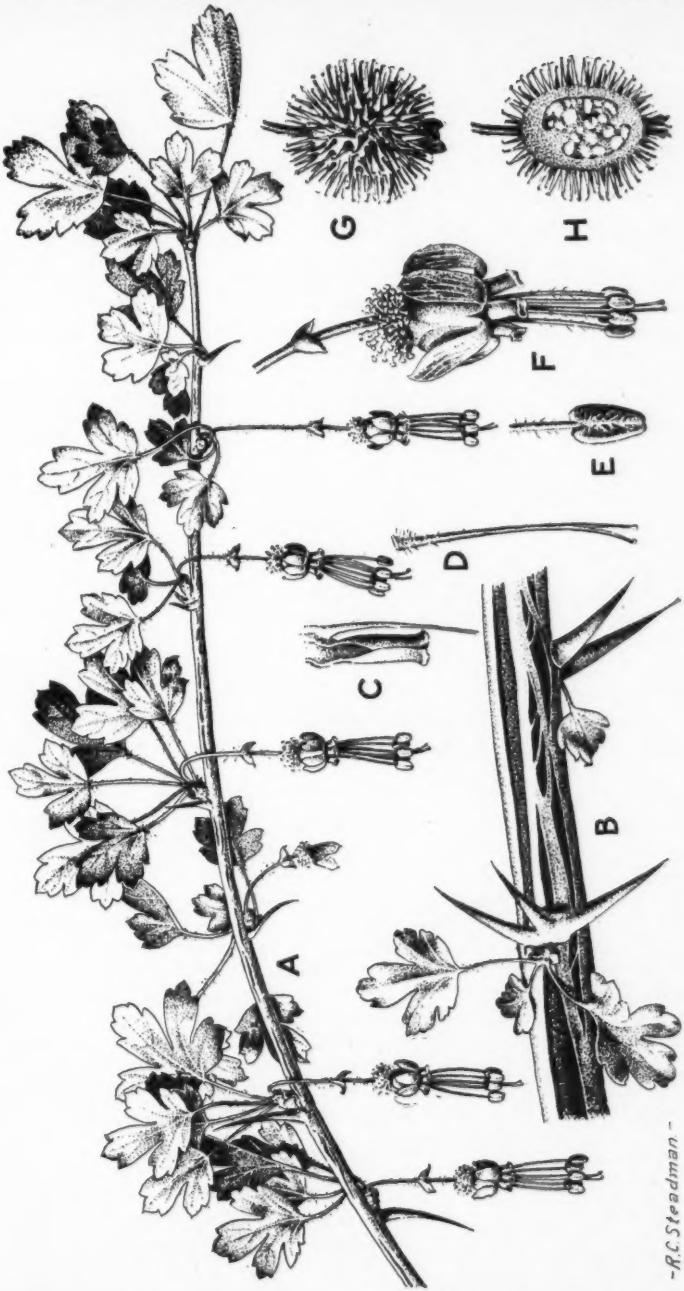
D.—Style. ×2.

E.—Anther, from the back. ×4.

F.—Flower, with pedicel, bracts, and portion of the peduncle. Note the hairy filaments, the tubular petals, the reflexed sepals, and the gland-tipped hairs of the ovary, which develop into the prickles of the fruit. ×2.

G.—Immature berry, picked April 7. Natural size.

H.—Lengthwise section of an immature berry. Natural size.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FOWL TYPHOID, ITS DISSEMINATION AND CONTROL[1]

By B. F. KAUPP, *Poultry Investigator and Pathologist*, and R. S. DEARSTYNE
North Carolina Agricultural Experiment Station

INTRODUCTION

The epiornithological problems in the control of diseases of birds closely parallel the epidemiological problems in human beings. Intensive production of poultry in recent years has increased the problems of disease control, close study applied to this subject in the past few years bringing out the fact that even the closest system of quarantine leaves unexplainable loopholes for the spread of contagious poultry diseases.

Fowl typhoid undoubtedly stands out among the septicaemic diseases of the domestic fowl as a particularly virulent contagion. It is distributed throughout the various countries of the world, recognized as a particularly virulent disease caused by the *Bacterium sanguinarium* or *gallinarium* (*Eberthella sanguinaria* Moore), or, on the Continent, Klein's bacillus. As far back as 1888, Klein, in England, made an investigation of this disease, with such concise work as to have this specific bacterium known by his name in Europe. In 1895, Moore investigated the disease in the United States, calling it Infectious Leukemia of Fowls, and the causative organism the *Bacterium sanguinarium*. Since that time, the disease has been reported and investigated by various authorities.

MORPHOLOGY

The fowl typhoid bacterium is a short, thick rod, occurring singly or in pairs, measuring 1 to 2 microns by 0.5 micron; stains peripherally; nonspore forming; Gram-negative, staining readily with most stains but best with fuchsin; nonmotile.

CULTURAL CHARACTERISTICS

Growth on agar slant beaded, abundant, raised, smooth, opaque in 24 hours; agar colonies raised, entire, circular, and finely granular; optimum temperature 37½° C.; optimum acidity of medium P_H 6.4, although the organism has a high tolerance to organic acids, suitable growth being obtained on agar acidified with formic, malic, and oxalic acids between the ranges of P_H 4.8 and P_H 7.0; does not liquefy gelatin; nonchromogenic; heavy nitrate reduction in nitrate broth and on nitrate agar without gas; aerobic growth in glucose agar shake; slight production of hydrogen sulphid on surface of acetate agar; growth on potato fairly abundant and yellow-brown in color. There is a slight production in some instances of indol with the Salkowski test, but no reaction was obtained with the vanillin test, nor with Ehrlich's method; slight diastatic reaction takes place on starch agar; over 50 strains studied in 16 carbohydrates and higher alcohols showed no gas production at 37½° C. for 5 days. Acid is produced in

[1] Accepted for publication Dec. 6, 1923.

all sugars with the exception of lactose, which remains neutral, with a slight tendency toward alkalinity; milk slightly acidified in 5 days. The thermal death point is 62½° for 10 minutes; the bacterium lives in both distilled and tap water in the dark for over 20 days, but is killed in the same medium in the sunlight in less than 24 hours; on glass rods the organism retains its vitality in the dark for up to 89 hours, but loses it in the sunlight in less than 30 hours; resists dry heat to the extent of giving good growth when subjected to 75° C. for 5 minutes, but fails to give growth when subjected to the same temperature for 10 minutes. Killed in dilution 1:1,000 phenol, $HgCl_2$, 1:20,000. Stock cultures show decided loss of virulence after being transferred several generations on artificial media.

ARTIFICIAL INFECTION

Studies on 38 cases of typhoid, artificially infected per os, by subcutaneous inoculation, or through the drinking water, show the incubation period to be

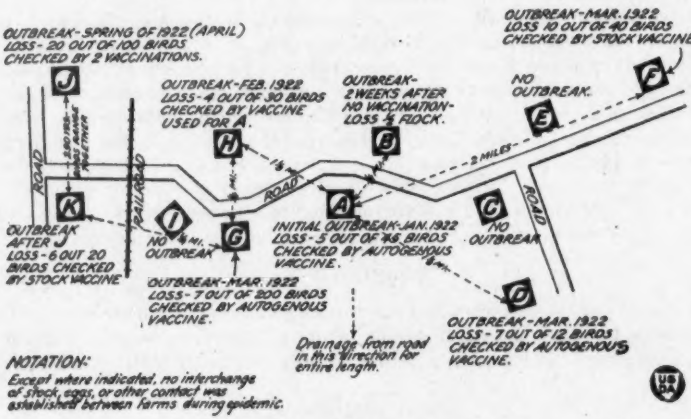

FIG. 1.—Map of region in which eight outbreaks of fowl typhoid occurred in the winter and spring of 1922.

from four to six days before definite clinical symptoms appear. Temperature in severe cases runs as high as 111.5°, respiration going as low as 23. The organism was recovered from the blood current of infected birds in two instances, four days after infection and six days after infection. In one instance the organism recovered was used as an infecting agent of another bird, this bird dying, while the original host recovered, having a normal laying rate until accidentally injured. There is a slight formation of agglutinins during the early part of the disease, demonstrated by Widal, and macroscopic agglutination tests in low dilutions. Hematological studies show a reduction of erythrocytes; leucocytosis, with a decided increase in the polymorphonuclear leucocytes. There is a decided lack of coagulability of the blood. The polymorphonuclear leucocytes may run as high as 95 per cent while the lymphocytes go down to 5 per cent; the erythrocytes fall as low as 1,160,000; the face, comb, and wattles become anemic; hemoglobin may fall to 75 per cent.

CLINICAL STUDIES

In the course of investigation of this disease, the writers were consulted on numerous outbreaks in the territory adjacent to the institution. The outbreaks were marked by exceptional severity of infection, with an ensuing high death rate. In these outbreaks the disease usually affected the adults. Clinically the symptoms are not pronounced at first, the birds being dull, sleepy, showing a loss in appetite, with marked increase in thirst. There is a rise in temperature with a sulphurous discharge from the bowel which sometimes is whitish mucoid in character. Death is usually preceded by partial loss of use of limbs, dispnoea, subnormal temperature and profuse diarrhea. The duration of the disease depends on the severity of infection and the natural resistance of the bird. Some of the birds doubtless recover, to become carriers through virulent bacilli voided in the dejecta.

GROSS AND MICROSCOPIC ANATOMY

Post mortem examinations show rigor mortis soon after death. The comb, face, and visible mucous membranes may be anemic. Serous effusion may be

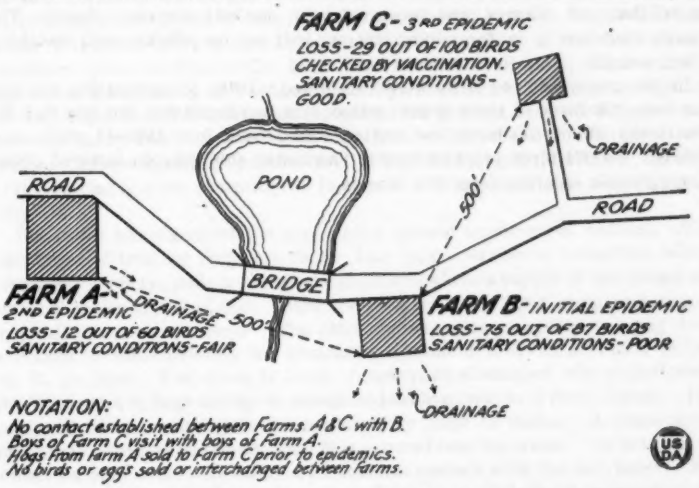

FIG. 2.—Map of region in which three outbreaks of fowl typhoid occurred.

observed around the heart and liver; hydropericardium may be present; the heart is congested and has a parboiled appearance; microscopically congestion is present, some of the muscle fibres have lost their cross striation, and cloudy swelling exists. The gross appearance of the liver shows enlargement, friability, dark-red in some cases, areas of focal necrosis exist, and blood drips from the sectioned surface; microscopically both active and passive congestion is present, areas invaded by cells of inflammation indicating hepatitis; cloudy swelling present; some areas show groups of cells losing their nuclei and nearing focal necrosis. The kidneys appear swollen, the sectioned surface is graying in color, narrow red streaks are observed over the surface, indicating congestion; microscopically both active and passive congestion is present. Areas in which cells are losing their nuclei and nearing a state of focal necrosis are seen; glomerulitis may be present. The spleen is enlarged, dark, and sometimes mottled. The lungs are normal. *Eberthella sanguinaria* may be recovered from the heart, liver, spleen, kidneys, lungs, ovaries or testicles according to sex, bone marrow, and brain.

EPIORNITHOLOGICAL STUDIES

As to the foci of infection, field studies in cases of well-defined epidemics have revealed in each case a starting point, with a definite advance of the disease. No conveying agent could be found, as only in rare instances could the investigators establish such contact between the locations of outbreaks as would be a factor in transmitting the disease. The possibility that English sparrows were the conveying agent is under investigation at the present time. Figures 1 and 2 show graphically the history of two typical epidemics of fowl typhoid. The progressive advance of the disease may be clearly traced.

METHOD OF CONTROL

The most practical method of treating this disease is vaccination, using autogenous vaccines when possible, and stock vaccines when the former are not procurable. The same principle of desensitization as in making other vaccines is used in preparing fowl typhoid vaccine, 24-hour bacillary saline emulsion being heated one hour at 60° C., tested for efficiency of desensitization by pig inoculation and cultures, and preserved with one-half per cent phenol. The dosage used was 1 cc. for adults and one-half cc. for chicks, each weighing 1 to 2 pounds.

In 19 epidemics 2,140 birds were vaccinated. Prior to vaccination the loss had been 303 birds in these flocks; subsequent to vaccination the loss was 41, practically all of the birds lost having well-defined fowl typhoid when vaccinated. Of 974 birds prophylactically vaccinated this year on infected premises, no losses occurred from this disease.

EFFECTS OF THE MODIFIED HOT-WATER TREATMENT ON GERMINATION, GROWTH, AND YIELD OF WHEAT[1]

By V. F. TAPKE[2]

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INTRODUCTION

The loose smut of wheat (*Ustilago tritici* (Pers.) Jens.) (Pl. 1), according to estimates compiled in the Plant Disease Bulletin [3] for the years 1917 to 1921, inclusive, caused an average annual loss to farmers of the United States amounting to more than 11,000,000 bushels. Recommendations for control of this disease in this country have been confined to treatment of the seed by the modified hot-water method. In comparison with other standard cereal-seed treatments, however, this method is considerably more tedious to apply and also requires more labor and equipment, facts which doubtless help to explain why its adoption by the individual farmer has been limited. In 1918, R. G. East, county agricultural agent in Shelby County, Ind., in an attempt to make the modified hot water treatment more practicable, established a central or community seed-treatment plant at Shelbyville, Ind. The Division of Botany, Department of Agricultural Extension, of Purdue University, the Office of Cereal Investigations of the United States Department of Agriculture, and the Farmer's Association of Shelby County, Ind., cooperated with Mr. East in this endeavor. The community system became instantly popular and within two years, according to Pipal (*10*)[4], 20 plants were operating in Indiana. A few plants also were in use in Virginia.

Pipal (*10*) has described the community system employed in Indiana, where two types of treating plant are used. One type operates in connection with a creamery, cannery, mill, or other establishment where a supply of live steam and suitable tanks are available. Here the farmers bring their grain and treat it in small quantities in sacks. The other type employs a special treating drum and tank for administering the 10-minute treatment, a device first used in 1918 by R. G. East. The drum is built of heavy, small-meshed wire or perforated sheet iron and is large enough to accommodate five bushels of soaked grain. It is equipped with a hoisting device operated by hand or motor. A crank also is provided to revolve the drum when it is lowered into the water. As it turns, the wheat tumbles over, thus insuring its uniform contact with the hot water. Following the treatment, the grain is spread out to cool; later it is resacked and returned to the farmer. In order to defray the expenses of the community plant

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[2] The writer makes grateful acknowledgment to Drs. W. H. Tisdale, H. B. Humphrey, and C. R. Ball for helpful criticisms of the manuscript, and to the agronomists in the wheat projects in the Office of Cereal Investigations for supplying many of the seed samples used in the experiments.

[3] U. S. Department of Agriculture, Bureau of Plant Industry, Plant Disease Survey. ESTIMATE OF CROP LOSSES DUE TO PLANT DISEASES, 1917. WHEAT. U. S. Dept. Agr. Bur. Plant. Indus. Plant. Dis. Bul. *2*: 3-4. 1918. (Mimeographed.)

——— CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1918-1921. WHEAT. U. S. Dept. Agr. Bur. Plant. Indus. Plant. Dis. Bul., Suppl. *6*: 190-191; *12*: 309-310; *18*: 318-319; *24*: 490-491. 1919-1922. (Mimeographed.)

[4] Reference is made by number (italic) to "Literature cited," p. 97.

a charge is made, based on actual cost of operation and equipment. The rate usually approximates 25 cents a bushel.

Table I, adapted from Pipal (*10*), shows the increase in number of community plants for applying the hot-water seed treatment in Indiana, and in the quantity of grain treated from 1918 to 1920.

TABLE I.—*Increase in the number of community plants for applying the hot-water seed treatment in Indiana and in the quantity of grain treated from 1918 to 1920*

Year	Type and number of treating plants			Bushels of wheat treated
	Using sacks in connection with a creamery, etc.	Using a treating drum	Total	
1918	0	1	1	616
1919	3	5	8	3,419
1920	11	9	20	9,687

This growth in the use of the modified hot-water treatment brought forth insistent inquiries concerning the effects of treatment on the germination, growth, and yield of wheat. Interviews with patrons of the community plants revealed that there was some apprehension regarding the amount of injury resulting from treatment. The available literature did not fully answer the questions and the investigations reported herein were undertaken to provide the desired information.

METHODS AND MATERIALS

In the following experiments the seed was treated in cheesecloth bags with ample room to allow for swelling of the grain. A 60-gallon tank, used for treatment, was equipped with two motor-driven propellers which kept the water thoroughly agitated and at a uniform temperature. Thermostatically controlled electric heaters were employed to maintain the temperature of the 10-minute bath, which was held at 54° C. in all cases with a plus or minus variation not in excess of 0.2°. All of the thermometers employed had been standardized by the United States Bureau of Standards. In applying the modified hot-water treatment the wheat was (1) presoaked for 4 to 5 hours in cold water, (2) dipped momentarily in water at about 49°, and (3) immersed in water at 54° for 10 minutes. Immediately after treatment the seed was spread in a thin layer to cool, and in order to reduce its moisture content to about that of the untreated seed, it was left to dry for 5 days or more at room temperature. Soil-germination tests in the greenhouse were made by sowing 100 seeds per flat 1 x 2 feet. Uniform spacing and depth of sowing were insured by pressing into the soil 100 one-inch pegs inserted in a board equidistantly. The kernels were dropped into the holes so made and then covered.

EFFECT OF THE MODIFIED HOT-WATER TREATMENT ON GERMINATION

Difficulties involved in making germination tests in the field throughout the year led to a study to determine whether or not tests in moist blotters or in soil in flats in the greenhouse could be substituted. Preliminary germination tests in moist blotters showed that many of the treated seeds germinated more or less abnormally. For instance, some of the primary rootlets would be stunted or absent, or only a plumule or a radical, or aborted forms of one or both would

appear. However, some of the seedlings which were abnormal at the end of the 5-day period usually allotted for the germination test assumed a normal appearance later on. In several instances 11 days were required for the germination of treated seeds to reach the stage commonly attained by untreated seeds on the fifth day. It was difficult or impossible to know whether or not seeds producing these types of seedlings in the blotters would produce plants if sown in the soil. An experiment was conducted, therefore, in order to study the effects of different periods of incubation on the percentage of germination in blotters and to compare the results from each period of incubation with those obtained from tests in soil in the field and in flats in the greenhouse. Five varieties were used, namely, Brown Winter Fife (C. I. 1933),[5] Currell (C. I. 3326), New Amber Longberry (C. I. 1973), Purplestraw (C. I. 1915) and Goens.[6] The Goens seed lot was grown in Indiana in 1921. The other varieties were grown on Arlington Experiment Farm, Rosslyn, Va., in 1921. A part of the seed of each of the varieties was treated on October 10, 1921, and dried 7 days at room temperature. Thereafter, 200 untreated and 200 treated seeds of each variety were taken for each of the soil tests and for the blotter test. All sowings were made on October 17, 1921. After 30 days germination counts were made in greenhouse and field and average percentages of germination for the 5 varieties were determined as follows:

Germination in soil in the greenhouse: Untreated seed 88.8 per cent; treated seed 66.9 per cent.

Germination in soil in the field: Untreated seed 89.2 per cent; treated seed 66.5 per cent.

A close agreement between field and greenhouse results is indicated. The results of the blotter test are presented in Table II. The numbers recorded at each germination period included only those seeds which appeared to germinate normally.

TABLE II.—*Average percentages of germination of 200 untreated and 200 treated seeds of each of five wheat varieties sown in moist blotters*

Treatment of seed	Varieties	Average percentage of germination after incubation for the following number of days:				
		3	5	7	9	11
Untreated	Brown Winter Fife	39.0	95.5	96.5	97.0	97.0
Do	Currell	53.0	96.5	98 0	98.0	98.0
Do	Goens	38.0	95.5	96 0	96.0	96.0
Do	New Amber Longberry	25.0	94.0	95.5	95.5	95.5
Do	Purplestraw	30.0	93.0	96.5	96.5	96.5
Average		37.0	94.9	96.5	96.6	96.6
Treated by the modified hot-water method.	Brown Winter Fife	0.5	63.0	89.0	90.5	91.5
Do	Currell	3.0	60.5	91.5	92.0	93.0
Do	Goens	2.5	64.5	85.5	86.0	86.0
Do	New Amber Longberry	0	49.5	82.5	84.5	84.5
Do	Purplestraw	1.5	61.0	87.5	88.0	88.5
Average		1.5	59.7	87.2	88.2	88.7

Table II shows that none of the periods of incubation yielded germination percentages which compared favorably with those obtained from seed sown in the soil. Treated seed, incubated seven days in the laboratory, germinated over 20 per

[5] Accession numbers of the Office of Cereal Investigations.

[6] Goens wheat is extensively grown under its synonym, Red Chaff, in sections of Indiana where loose smut is very prevalent. It is unusually susceptible to loose smut, and perhaps more than any other variety is subjected to modified hot-water treatment. For these reasons it has been included in many of the experiments reported herein.

cent higher than treated seed sown in the soil. Evidently, many of the retarded or abnormal seedlings which survive on the blotter would not survive in the soil. The reliability of the test in greenhouse soil when compared with a field-soil test was confirmed in further trials and therefore was adopted in the germination studies which follow.

The effects of the modified hot-water treatment on germination were studied first on 33 varieties of wheat, 15 of which were grown on Arlington Experiment Farm, Va., and 18 at Chico, Calif., in 1921. A small portion of each of the varieties was treated on December 12, 1921, and dried six days at room temperature, after which 100 untreated and 100 treated kernels of each variety were sown in soil in greenhouse flats on December 18, 1921. Germination counts were made one month later and the results are presented in Table III. In this and other tables in which the wheat varieties are classified, the classification of Clark, Martin, and Ball (*3*) has been followed.

TABLE III.—*Percentages of germination of machine-threshed seed of 33 wheat varieties, untreated or treated by the modified hot-water method*

Variety	C. I. No.	Source	Percentage of germination	
			Untreated	Treated
Hard red spring wheats:				
Haynes Bluestem	2874	Chico, Calif.	92	44
Marquis	3641	do	86	60
Preston	3081	do	76	18
Durum wheats:				
Kubanka	1440	do	74	38
Monad	3320	do	77	46
Hard red winter wheats:				
Kanred	5146	do	93	40
Minturki	6155	do	77	12
Turkey	1558	do	92	47
Soft red winter wheats:				
Fulcaster	6162	Arlington Experiment Farm, Va.	90	53
Bearded Purplestraw	1911–1	do	94	57
Diets	1981	do	97	70
Lancaster	1945	do	88	69
Stoner	2980	do	72	54
Fultz	3598	do	88	57
Harvest Queen	4882	do	92	27
Leap	4823	do	94	64
Mammoth Red	2008	do	87	57
Mediterranean	1930	do	88	60
Michigan Amber	4864	do	93	81
Minhardi	5149	Chico, Calif.	90	40
Poole	3489	Arlington Experiment Farm, Va.	92	61
Red Rock	5976	do	73	40
Shepherd	6163	do	93	67
White wheats:				
Baart	1697	Chico, Calif.	95	57
Dawson	6161	Arlington Experiment Farm, Va.	93	66
Dicklow	3663	Chico, Calif.	92	51
Federation	4734	do	94	60
Genesee Giant	1744	do	77	62
Hard Federation	4733	do	92	76
Hybrid 128	4512	do	87	38
Little Club	4066	do	89	39
Pacific Bluestem	4067	do	93	69
Regenerated Defiance	3703	do	80	60
Average			87.6	52.7

Table III shows that the treated wheat in this experiment was injured severely, germinating 34.9 per cent less than the untreated, on an average.

During the experiment, it was observed that seed coats in many of the varieties were badly broken, and showed unmistakable signs of severe threshing injury. In this connection it is noteworthy that over 50 years ago Nobbe (*7*) pointed out that the unbroken seed coat is very effective in protecting the embryo

against injury from copper sulphate. He found that machine-threshed grain, on this account, was far more susceptible to treatment injury than grain threshed by hand. Kühn (*6*), Von Tubeuf (*13*), Volkart (*14*), Burmester (*2*), Hurd (*5*), and others have confirmed Nobbe's work. A test was made, therefore, to determine whether the physical condition of the seed coat might be an important factor also in connection with seed injury by the modified hot-water treatment. Three hand-threshed wheat varieties, grown on Arlington Experiment Farm in 1921, were used. Four lots, each containing 100 kernels, were taken from each variety and prepared as follows: Lot 1, seed coats left unbroken, seed untreated; lot 2, seed coats left unbroken, seed treated; lot 3, seed coats slit over the embryo, seed untreated; lot 4, seed coats slit over the embryo, seed treated. (The seed coat may be slit easily with a sharply pointed needle after first immersing the kernels in water for several minutes.) The modified hot-water treatment was applied simultaneously to lots 2 and 4 on January 25, 1922, after which they were dried six days at room temperature. The untreated and treated seed was sown then in soil in the greenhouse on January 31, 1922. One month later the seedlings were counted. The results are presented in Table IV.

TABLE IV.—*Percentages of germination of hand-threshed seed of three wheat varieties with seed coats unbroken or broken over the embryo, untreated or treated by the modified hot-water method*

Variety	C. I. No.	Percentage of germination			
		Seed coat unbroken		Seed coat broken over embryo	
		Untreated	Treated	Untreated	Treated
Fulcaster	3115-2	100	100	100	6[a]
Dawson	1733	98	100	98	4[a]
Diehl-Mediterranean	1395	96	94	97	3[a]
Average		98.0	98.0	98.3	4.3

[a] Seedlings very small and spindling.

As indicated in Table IV, the hot-water treatment reduced germination only when the seed coats were broken. The physical condition of the seed coat over the embryo, therefore, bears a definite and important relation to the amount of injury caused by the modified hot-water treatment (Pl. 2).

In many samples of machine-threshed wheat, breaks in the seed coats frequently were found over the endosperm only. In order to determine the effects of hot-water treatment on grain with seed coats in this condition, four lots of 200 kernels each from a hand-threshed sample of the variety China (C. I. 180) were used. The seed coats were left unbroken in the first lot. In the second lot, a small slit was made in the coat about one-eighth of an inch above the top of the embryo. In the third lot, a small part of the seed coat was scraped away at the brush end of the seed. In the fourth lot, a slit was made in the seed coat over each cheek in a location behind the embryo. One hundred kernels of each of the four lots were treated simultaneously on September 4, 1922, and dried 5 days at room temperature. The untreated and treated seed was then sown in soil in greenhouse flats on September 9, 1922, and the seedlings were counted after periods of 10, 20, and 30 days. Many which developed from the treated seed with the coats broken over the endosperm were very small and spindling. For this reason, the seedlings were recorded as either normal or abnormal at each of the three periods when the counts were taken. The results are presented in Table V.

TABLE V.—*Percentages of normal, abnormal, and total germination of untreated and treated seeds of China (C. I. 180) wheat with seed coats unbroken or broken at definite locations over the endosperm*

Number of days after sowing	Percentage and character of germination															
	Seed coats unbroken				Seed coats broken over endosperm											
					¼ inch above embryo				On brush end				On each cheek behind embryo			
	Untreated		Treated		Untreated		Treated		Untreated		Treated		Untreated		Treated	
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
10	97	0	93	0	99	0	15	40	95	0	9	15	98	0	2	3
20	97	0	96	0	99	0	15	49	95	0	9	26	98	0	4	7
30	97	0	96	0	99	0	15	51	95	0	9	27	98	0	4	8
Total germination	97		96		99		66		95		36		98		12	

Table V shows that when the seed coats are broken over the endosperm there is a severe reduction in normal and total germination (Pl. 3). In the light of these data, the original supply of the 33 varieties listed in Table III was examined carefully for broken seed coats. It was found that the lots most severely injured by treatment invariably showed a high percentage of seed-coat breakage.

During the course of some of the preceding experiments it was observed that hand-threshed kernels with unbroken seed coats germinated more slowly when the seed was treated. A typical example is shown in Table VI for Leap (C. I. 4823) wheat. Five hundred hand-threshed kernels were treated on September 1, 1922, and dried seven days at room temperature, following which, the treated kernels and a like number of untreated from the same lot were sown on September 8, 1922, in soil in greenhouse flats. All of the seed was sown and germinated under like conditions. From the date of emergence of the first seedling daily records of the number of seedlings which had emerged were taken for one month. The results are presented in Table VI.

TABLE VI.—*Rate of emergence of seedlings from 500 untreated and 500 treated kernels of hand-threshed Leap (C. I. 4823) wheat*

Treatment of seed	Percentage of emergence after the following number of days						
	5	6	7	8	9	10	30
Untreated	85	98	98	98	98	98	98
Treated by the modified hot-water method	0	22	64	79	96	98	98

Table VI shows that the modified hot-water treatment materially affects the germination of seeds with unbroken coats, although it may produce no actual mortality (Pl. 4, A, B, E, and F). Atwood (*1*) has noted the possibility that concentrations of formaldehyde which do not materially injure germination percentages may materially disturb the physiologic processes related to germination and subsequent growth.

It is noted thus far that the action of the modified hot-water treatment on germination may range from retardation without reduction to outright killing of practically 100 per cent of the seed, depending entirely upon the physical condition of the seed coat. In actual practice seed-coat damage results mainly from threshing. An indication of the amount of possible seed injury traceable to the modified hot-water treatment following threshing injury to seed coats has been obtained from 25 samples of the crop of 1922. Twenty of them came from Hancock, Rush, and Shelby Counties, Ind., where loose smut usually occurs abundantly, and where the hot-water treatment is used extensively. Each of the 25 seed lots consisted of both machine-threshed grain and grain in the head taken from the field on the day of threshing. The grain in the head was carefully threshed by hand. One hundred kernels of each of the 25 different lots of hand-threshed and of machine-threshed seed were treated simultaneously, on October 14, 1922. The grain then was dried six days at room temperature and sown on October 20 in soil in greenhouse flats at Arlington Experiment Farm. One hundred untreated hand-threshed kernels and 100 untreated machine-threshed kernels of each seed lot were sown in similar flats as controls. The seedlings were counted on November 14, one month after sowing. The results are presented in Table VII and in Plate 4.

TABLE VII.—*Percentages of germination of wheat showing the effects of the modified hot-water treatment on machine-threshed and hand-threshed seed of 25 different lots of seed from nine varieties as compared with untreated controls*

Class and variety	Source	Seed lot	Percentage of germination			
			Machine-threshed		Hand-threshed	
			Untreated	Treated	Untreated	Treated
Soft red winter wheats:						
Fultz	Indiana	A	85	61	99	95
Do	do	B	96	68	96	95
Goens (Red chaff)	do	C	90	40	98	95
Do	do	D	99	71	98	98
Do	do	E	89	58	100	100
Do	do	F	94	62	98	99
Do	do	G	90	52	99	97
Do	do	H	81	49	99	99
Do	do	I	88	64	99	99
Do	do	J	89	52	96	96
Do	do	K	71	45	99	97
Do	do	L	85	55	100	98
Do	do	M	90	51	99	98
Leap	Virginia	N	78	39	97	97
Poole	Indiana	O	87	54	97	96
Do	do	P	93	64	98	96
Do	do	Q	87	45	100	99
Red May (Michigan amber)	do	R	93	77	99	100
Do	do	S	90	65	100	96
Do	do	T	99	77	99	100
Do	do	U	89	61	100	93
White wheats:						
Defiance	Idaho	V	91	53	100	99
Dicklow	do	W	84	34	100	97
Hard Federation	do	X	84	61	82	83
Little Club	California	Y	86	57	96	99
Average			88.3	50.6	97.9	96.8

Table VII shows a reduction of 31.7 per cent in the germination of the treated machine-threshed seed, while the treated hand-threshed seed was reduced only 1.1 per cent. This difference of 30.6 per cent between the germination of machine-threshed and hand-threshed seed shows clearly the relation of mechanical injury of the seed coat and possible subsequent injury from application of hot water (Pl. 4).

Having shown before that unbroken seed coats protect the grain from treatment injury, it may be asked why treated hand-threshed wheat (Table VII) did not germinate fully as well as untreated hand-threshed seed. It is possible that some injury to seed coats may result even from hand threshing. Also, localized imperfections too slight even to be seen microscopically, may occur in seed coats. Shull (*12*) found such a condition to be frequent in the seed coats of Xanthium. Hurd (*5*) observed that wheat kernels with apparently unbroken seed coats occasionally were injured when treated with copper sulphate.

In an experiment to determine if a break in the seed coat invariably could be detected microscopically and thus enable the observer to predict the amount of injury likely to result from treatment, a machine-threshed sample of Haynes Bluestem (C. I. 2874) was employed. The untreated seed germinated 95 per cent. One hundred kernels selected at random and treated, germinated 12 per cent, while a like number of kernels microscopically examined, selected for unbroken seed coats, and treated simultaneously with the above, germinated 74 per cent. This indicates that it is hardly possible to detect all of the breaks in a seed coat even under the microscope, and that the small fractures which escape detection may result fatally to the embryo through injury from treatment. Wallden (*15*) was able to detect broken seed coats by the use of eosin, which does not penetrate the unbroken seed coat, but enters every small fissure, staining the seed tissues at the point of entrance.

THE NATURE OF SEED-COAT PROTECTION FROM HOT-WATER INJURY

The important rôle of the seed coat in protecting the embryo from injury during the hot-water treatment led to an investigation of the manner in which it functions. Obviously, this protective action must be exerted either in the 4 to 6-hour presoak in cold water, in the 10-minute bath in water at 54° C., or in both. In an experiment to determine the point, three lots of 50 hand-threshed kernels of each of the three varieties, Leap (C. I. 4823), Poole (C. I. 3489), and Power Fife (C. I. 3697) were used. In the first lot the seed coats were left unbroken through both the presoak and 10-minute baths. In the second lot the seed coats were broken over the embryo before the 10-minute bath. In the third lot the seed coats were broken over the embryo before both baths. The presoak bath for 5 hours at 20° C. was applied to all lots simultaneously on February 10, 1923, and each lot was weighed carefully before and after presoaking to determine the possible influence of broken seed coats on the amount of water absorbed by the seed. All lots were treated then simultaneously for 10 minutes at 54° C., dried 6 days at room temperature, and then sown on February 16, 1923, in soil in greenhouse flats. One month later, on March 16, the seedlings were counted. The results are presented in Table VIII.

TABLE VIII.—*Effect of the presoak bath or 10-minute bath, or both, on wheat with seed coats unbroken or broken during the 10-minute bath or throughout treatment*

Variety	C.I.No.	Actual and average percentage of germination when seed coats were:		
		Unbroken throughout treatment	Unbroken during presoak bath; broken during 10-minute bath	Broken throughout treatment
Leap	4823	81.0	14.0	0
Poole	3489	98.0	34.0	0
Power File	3697	100.0	24.0	0
Average		93.0	24.0	0
Average percentage of increase in weight at end of presoak bath		23.09	23.04	27.65

Table VIII shows that germination was increased from zero to 24 per cent when seed coats were unbroken during the presoak and from 24 to 93 per cent when seed coats were unbroken throughout treatment. Unbroken seed coats, therefore, effect protection from injury during both baths of the modified hot-water method and particularly during the 10-minute treatment. Table VIII also shows that the amount of water absorbed by the seed was greater in the presence of seed coats broken during the presoak bath.

According to Osborne (*8*) leucosin is the only one of three proteins in the wheat embryo affected by a temperature of 54° C. A solution of leucosin (after a 4 to 6-hour presoak, the leucosin of the embryo undoubtedly is in solution) becomes turbid at 48°., and forms a flocculent coagulum at 55°. The hot water at 54°., therefore, through direct contact with the embryo, probably coagulates some of the leucosin when the surrounding seed coat is broken, and thus may bring about the lethal action previously noted.

An examination of its physical structure shows how the seed coat may function to insulate the embryo. According to Percival (*9*) and others, the outermost membrane of the seed coat over the embryo is the pericarp, and the inner membranes successively are the testa and nucellar layer. It was found that the presence or absence of the pericarp did not influence the germination of hand-threshed treated seeds. The pericarp was removed [7] from 100 hand-threshed kernels of Fulcaster (C. I. 6162). A like number of kernels from the same lot with unbroken pericarps were employed. The two lots of seed were subjected to the hot-water treatment simultaneously and each was sown in soil in greenhouse flats. The resulting germination was 100 per cent for each lot. Hurd (*5*) found that the presence or absence of the pericarp also had no influence on the germination of hand-threshed seeds treated with copper sulphate. The burden of protection, therefore, falls upon the testa or the nucellar layer, or both. According to Percival (*9*), the crushed cells of these membranes in the ripe seed together form a layer six cell-walls thick over the embryo, and it seems not unreasonable that a structure of this nature might afford protection to the extent noted during the 10-minute treatment period.

Table VIII also shows that some protection was afforded by unbroken seed coats during the presoak, and that the rate of water absorption by the seed during the presoak was greater when the seed coats were broken. In order to determine

[7] Soaking the seed in water for several minutes causes the pericarp to pucker and loosen, when it may be peeled off readily with the aid of a needle or scalpel.

the relationship between water content of the seed and injury by treatment in the presence of broken and unbroken seed coats, several experiments were conducted. In the first, 8 lots each containing 100 hand-threshed kernels of the variety Haynes Bluestem (C. I. 2874) were used. In 5 of the lots the seed coats were broken over the embryo with a needle. Some of the lots were presoaked for varying periods, as shown in Table IX, and a record was made of the resultant increases in weight. The seed coats were undisturbed in three of the lots. All of the seed was subjected simultaneously to the 10-minute treatment at 54° C., on February 15, 1923, then dried five days at room temperature, and sown in flats in the greenhouse on February 20. The seedlings were counted on March 20 and the results are presented in Table IX and in Plate 5.

TABLE IX.—*Percentages of germination and increase in weight of hand-threshed seed of Haynes Bluestem wheat (C. I. 2874) with broken or unbroken seed coats, some presoaked during different periods prior to treatment with hot water*

Lot No.	Condition of seed coat	Length of presoak bath	Weight of seed before presoak bath	Weight of seed after presoak bath	Increase in weight	10-minute treatment at 54° C	Germination
		Hours	*Gm.*	*Gm.*	*Per cent*		*Per cent*
1	Broken over the embryo	0				Untreated	96.0
2	do	0				Treated	38.0
3	do	1	3.260	3.745	14.9	do	19.0
4	do	2	3.180	3.860	21.4	do	15.0
5	do	4	3.250	4.130	27.1	do	4.0
6	Unbroken	0				Untreated	95.0
7	do	8.5	3.370	4.510	33.8	Treated	96.0
8	do	10.5	3.380	4.660	37.9	do	93.0

Table IX shows (1) that in the absence of the presoak bath severe reduction in germination resulted from the 10-minute treatment when the seed coats were broken over the embryo (lot 2); (2) that injury increased with increase in duration of presoak and moisture content when the seed coats were broken over the embryo (lots 3, 4, and 5); and (3) that treatment did not impair germination appreciably when the seed coats were unbroken despite a high increase in water content (lots 7 and 8), (Pl. 5).

The relationship between increase in the duration of the presoak period and injury to seed with unbroken coats was studied further in the experiment which follows. Three lots each containing 50 hand-threshed seeds were prepared from each of four varieties of wheat. The three lots from each variety were presoaked 5, 10, and 15 hours, respectively, on February 18, 1923, then all were treated simultaneously in the 10-minute bath at 54° C., dried at room temperature for one week, and sown on February 25 in soil in greenhouse flats. The germination counts were made after 10, 15, and 30 days. The results are presented in Table X.

TABLE X.—*Percentages of germination of three lots of 50 hand-threshed seeds of each of four varieties of wheat presoaked for 5, 10, and 15 hours, respectively, prior to treatment for 10 minutes in water at 54° C.*

Variety	C. I. No.	Percentage of germination after:								
		10 days			15 days			30 days		
		Hours presoaked			Hours presoaked			Hours presoaked		
		5	10	15	5	10	15	5	10	15
Forward	6691	94	80	30	96	92	74	96	96	78
Goens		80	28	2	84	58	26	84	72	32
Illini Chief	5406	100	82	38	100	96	64	100	98	80
Stoner	2980	88	38	26	92	64	68	92	70	78
Average		90.5	57.0	24.0	93.0	77.5	58.0	93.0	84.0	67.0

Table X shows that even in the presence of unbroken seed coats increases in the duration of presoaking slowly result in increased retardation and a further reduction in germination.

EFFECTS OF THE MODIFIED HOT-WATER TREATMENT ON GROWTH

The retardation in germination and emergence of seedlings from kernels which survive treatment has been noted. The effects of treatment on seedling growth also were studied. Five lots of 100 seeds each were prepared, three lots being Goens (Red Chaff) and one lot each being Fulcaster and Purplestraw. Fifty seeds from each lot were subjected to modified hot-water treatment on October 14, 1921, and then dried for one week. On October 21 all of the seeds were sown in soil in greenhouse flats. Eleven days later, on November 1, the height of each seedling was recorded. The results are presented in Table XI.

TABLE XI.—*Average height, 11 days after sowing, of seedlings from five lots of wheat grown from untreated seed and seed treated by the modified hot-water method*

Variety	Average height of seedlings in inches 11 days after sowing	
	Seed untreated	Seed treated
Goens	4.50	2.25
Do	4.75	2.25
Do	5.50	3.25
Fulcaster	5.00	4.00
Purplestraw	4.75	3.25
Average	4.90	3.00

Table XI shows that the seedlings from the treated half of the five lots averaged 1.9 inches shorter than the seedlings from untreated seed.

To facilitate a critical determination of the effects of the modified hot-water treatment on the growth of wheat from treated seed, studies were made of the effects of treatment on individual plants on (1) field germination, (2) overwintering, and (3) number of culms produced. The determination of the plant

yields included in the plan of experiment had to be abandoned. One hundred untreated and 100 treated machine-threshed kernels of each of the varieties, Fulcaster (C. I. 6162) and Purplestraw (C. I. 1915), grown on Arlington Experiment Farm, in 1921, were used. The treatment was applied on October 14, 1921, and the seed allowed to dry five days at room temperature. On October 19 the untreated and treated kernels of each variety were sown in the field in adjacent sets of 100 seeds, and each set consisted of three successive rows containing 33, 33, and 34 kernels, respectively. The kernels were spaced 7 inches apart in rows 1 foot apart.[8] One month after sowing, the seedlings were counted. In July, 1922, the number of plants which had matured and the average number of culms per plant were determined. The results are presented in Table XII.

TABLE XII.—*Effects of the modified hot-water treatment on the development of wheat plants from machine-threshed seed*

Variety	Seed untreated			Seed treated		
	Field germination	Plants overwintered and matured	Average number of culms per plant	Field germination	Plants overwintered and matured	Average number of culms per plant
	Per cent	*Per cent*		*Per cent*	*Per cent*	
Fulcaster	90.0	90.0	12.0	71.0	71.0	11.6
Purplestraw	79.0	79.0	19.3	76.0	76.0	19.1
Average	84.5	84.5	15.7	73.5	73.5	15.4

Table XII shows (1) that the field germination was somewhat less and that the number of culms per plant was slightly less from treated than from untreated wheat and (2) that plants from untreated and treated seed overwintered without reduction in numbers. The fact that the number of culms produced per plant was less from treated seed than from untreated seed suggests the possibility that the injurious effects of treatment on spaced plants may extend throughout their life, if we assume that the culms from treated seed did not produce heads of greater weight. Repetition of the experiment on a larger scale would be desirable, however, before drawing definite conclusions.

EFFECTS OF THE MODIFIED HOT-WATER TREATMENT ON YIELD

Experiments to determine the effects of the modified hot-water treatment on yield have been conducted for three years. On October 19, 1920, definite quantities of each of two lots of Goens (Red Chaff) wheat from different sources in Indiana were weighed and measured, treated, spread out in thin layers and left to dry for one week. At the end of this period the treated seed had regained its original weight and volume. On October 26, the untreated and treated seed of both lots was sown in fortieth-acre plats on Arlington Experiment Farm, Va., at the rate of 6 pecks per acre. The results are presented in Table XIII.

[8] Unpublished data by Dr. C. E. Leighty seem to indicate that at this distance the effect of competition by wheat plants is nil.

TABLE XIII.—*Loose smut infection and yield in 1921 of two lots of Goens wheat grown from untreated seed and from seed treated by the modified hot-water method*

Lot number	Acre yield in bushels		Percentage of loose smut	
	Untreated	Treated	Untreated	Treated
7	25.2	24.1	11	0
13	25.0	24.2	7	0
Average	25.10	24.15	9	0

Table XIII shows that plants from treated seed yielded 0.95 of a bushel per acre less than those from untreated, although the latter contained an average of 9 per cent loose smut.

In a yield test in 1922, there were used three different lots of Goens wheat grown in Indiana in 1921, and one lot each of Fulcaster (C. I. 6162) and Purplestraw (C. I 1915) wheats grown on Arlington Experiment Farm in 1921. A definite quantity of each lot was weighed and measured, treated on October 13, 1921, spread out in thin layers, and dried for one week. At the end of this period the treated seed had regained its original weight and volume. On October 20 the untreated and treated seed was sown in rod rows on Arlington Experiment Farm, Va., at the rate of 6 pecks per acre on the same land used for the yield experiments of the preceding year. Five plats, each divided into 2 sections, were used. Each of the 10 sections contained 55 rows, or 11 successive rows of each of the five seed lots. The first 9 rows of each seed lot comprised a study of other hot-water treatments not discussed in this paper, while the tenth and eleventh were sown to seed treated by the modified method and to the untreated seed, respectively. The results are presented in Table XIV.

TABLE XIV.—*Loose smut infection and yields in 1922 of five lots of wheat each grown from untreated seed and from seed treated by the modified hot-water method*

Section of plat	Lot No.	Variety	Acre yield in bushels of each of 5 plats										Average percentage of loose smut for all 5 plats	
			Plat 1		Plat 2		Plat 3		Plat 4		Plat 5			
			Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
													P. c.	*P. c.*
East	16	Goens	35.1	29.0	35.0	27.7	40.7	30.0	31.3	25.3	35.4	24.0	7.2	0
Do	17	....do	34.5	20.8	35.1	19.5	37.3	24.5	33.5	20.8	32.1	20.0	6.8	0
Do	19	....do	33.3	23.3	32.4	26.6	31.0	21.4	29.7	24.8	32.0	22.7	10.9	0
Do	26	Fulcaster	22.7	24.6	28.8	21.9	25.1	22.3	27.7	16.8	26.8	22.0	0	0
Do	27	Purplestraw	28.6	17.3	36.3	26.5	44.1	30.5	39.3	26.1	38.1	28.5	0	0
Average			30.8	23.0	33.5	24.4	35.6	25.7	32.3	22.8	32.9	23.4	.....	.....
West	16	Goens	32.1	28.7	36.3	26.8	40.5	29.3	38.1	33.6	35.3	34.3	7.1	0
Do	17	....do	36.0	23.8	40.1	31.6	39.5	25.6	39.7	25.7	34.5	24.3	11.1	0
Do	19	....do	31.1	28.6	29.7	28.3	32.8	33.9	32.1	27.3	28.0	21.1	15.0	0
Do	26	Fulcaster	32.0	32.0	28.0	29.4	31.8	33.3	34.8	32.7	31.4	24.3	0	0
Do	27	Purplestraw	30.6	27.4	34.6	31.0	34.8	35.7	36.6	29.7	37.0	32.2	0	0
Average			32.4	28.1	33.7	29.4	35.9	31.9	36.3	29.8	33.2	27.2	.....	.....

Average yield for all plats, east section: Untreated 33.0 bushels per acre; treated 23.9 bushels per acre.
Average yield for all plats, west section: Untreated 34.3 bushels per acre; treated 29.2 bushels per acre.
Average yield for all plats, both sections: Untreated 33.7 bushels per acre; treated 26.6 bushels per acre.

Table XIV shows that although the plants from the untreated seed contained an average of 5.8 per cent of loose smut, they outyielded those from the treated seed by 7.1 bushels per acre. The average from 7 seed lots in the two years shows that treated seed yielded 4 bushels per acre less than untreated, although plants' from the latter contained 7.4 per cent of smutted heads.

A comparison of the yields for the two years will show that reduction in yield from treated seed in 1922, amounting to 7.1 bushels per acre, is approximately 7.5 times the reduction of 0.95 bushels per acre in the yield from treated seed in 1921. It has been shown before (Tables IV and V) that the amount of seed injury resulting from the modified hot-water treatment is intimately related to the percentage of seed coats broken in threshing. Investigators have shown, moreover, that the amount of damage suffered by seed coats in the process of threshing is markedly greater in dry years than in wet years. Nobbe (*7*) first pointed out that the drier and more brittle the crop, the greater was the injury from threshing. Volkart (*14*) made similar observations and Wallden (*15*) also noted that in dry years the cracking of seed coats by threshing machines was more prevalent. In this connection, therefore, a study was made of the climatologic data for June and July, in 1920 and 1921, for the localities which supplied the seed used in the yield experiments in 1921 and 1922, respectively. The seed used was ripened, harvested, and threshed during June and July and the effects of weather conditions on the seed coats occurred in those months. The climatologic data are presented in Table XV.

TABLE XV.—*Temperature and rainfall during June and July, 1920 and 1921, in the localities in which was grown the seed wheat used in the yield experiments of 1921 and 1922, respectively*

County	State	Lot number	Year	Departures from normal			
				Temperature		Rainfall	
				June	July	June	July
				°F.	°F.	*Inches*	*Inches*
Hancock	Indiana	7	1920	−1.0	−1.7	+1.52	+1.29
Shelby	do.	13	do.	−1.0	−2.1	−0.61	0
Rush	do.	16,17	1921	+2.4	+4.9	−0.66	−0.10
Hancock	do.	19	do.	+5.9	+5.4	−1.12	+0.30
Arlington	Virginia	26,27	do.	+1.7	+2.4	−0.73	+0.14

Reference to Table XV shows abnormally low temperatures and a rainfall slightly above normal in June and July, 1920, in the localities which supplied the seed used in 1920–21. For the State of Indiana, as a whole, July, 1920, was one of the coolest on official record. In striking contrast, the temperatures were abnormally high and the rainfall below normal in June and July, 1921, in the counties supplying the seed used in 1921–1922. June, 1921, was the warmest on official record in Indiana and the average temperature of July, 1921, has been exceeded only twice in 34 years. It was the hottest July in Virginia since 1901. In the light of these data, the condition of the seed coats of wheat grown in 1920 and 1921 was examined carefully. It was very evident that the latter had sustained considerably more injury in threshing. It is interesting and noteworthy that the weather conditions, through their influence on the seed coats in withstanding threshing injury, may be potent factors in determining the amount of seed injury and subsequent yield which result from treatment of the seed by the modified hot-water method.

Attention is directed to the parts of Table XIV dealing with the average yields on the east and west sections of the plats. It will be noted that the

average yields per plat from both the untreated and treated seed were higher on the west than on the east sections, without exception. It is noteworthy, however, that the average yield of all the plats of the west section exceeded that of the east section by only 1.3 bushels per acre when the seed was untreated and by over four times this amount (5.3 bushels per acre) when the seed was treated. This shows a pronouncedly stronger reaction to less favorable soil conditions in plants from treated seed.

In the yield tests of 1923, part of the treated seed was sown at an increased rate to offset the loss in stand brought about by the treatment. About a month before the usual time of sowing (September 15, 1922) small quantities of each of two seed lots of Goens wheat from different sources in Indiana were treated and dried one week at room temperature. Subsequently, 500 untreated and 500 treated kernels of each seed lot were sown (September 22, 1922) in the field which was used for the later sowings. Three weeks later (October 13, 1922), germination counts were made with the following results:

Seed lot 31 from untreated seed, 87.0 per cent; from treated seed, 48.0 per cent.

Seed lot 35 from untreated seed, 84.5 per cent; from treated seed, 54.5 per cent.

In view of the fact that germination of both lots of treated seed was approximately 50 per cent, the rate of seeding was doubled in part of the sowings. Definite quantities of each of the two seed lots were weighed and measured, treated on October 18, 1922, spread out in thin layers and dried one week. At the end of this period, the treated seed had regained its original weight and volume. All of the untreated and part of the treated seed was sown then on October 25, in rod rows at the rate of 6 pecks per acre. Part of the treated seed was sown at the rate of 12 pecks per acre. In order to determine the effects of sowing freshly treated seed, a practice carried on by some of the patrons of the community seed-treating plants for the purpose of saving the labor and time involved in drying grain, seed from both lots was treated and sown wet at the rates of 6 and 12 pecks per acre, respectively, also on October 25. The necessary amount for each row was weighed and treated in a small gauze sack. The order of sowings in the plat was as follows in 10 successive replications.

Seed lot No. 31.—Seed untreated; sown dry; 6 pecks per acre. Seed treated; sown dry; 6 pecks per acre. Seed treated; sown dry; 12 pecks per acre. Seed treated; sown wet; 6 pecks per acre. Seed treated; sown wet; 12 pecks per acre.

Seed lot No. 35.—Seed untreated; sown dry; 6 pecks per acre. Seed treated; sown dry; 6 pecks per acre. Seed treated; sown dry; 12 pecks per acre. Seed treated; sown wet; 6 pecks per acre. Seed treated; sown wet; 12 pecks per acre.

The results are presented in Table XVI.

TABLE XVI.—*Loose smut infection and yields in 1923 of two lots of Goens wheat from untreated seed and seed treated by the modified hot-water method*

Lot No.	Acre-yield in bushels					Percentage of loose smut	
	Seed untreated	Seed treated					
	6 pecks per acre	6 pecks per acre		12 pecks per acre		Seed	
	Sown dry	Sown dry	Sown wet	Sown dry	Sown wet	Untreated	Treated
31	33.2	29.2	25.5	37.1	32.2	10.4	0
35	37.5	29.5	26.6	40.9	34.4	11.0	0
Average	35.4	29.4	26.1	39.0	33.3	10.7	0

Table XVI shows the following: (1) the yield from wheat treated by the modified hot-water method was less than that from untreated wheat when both treated and untreated seed were sown at the same rate. These results confirm those obtained by Freeman and Johnson (*4*) in 1908 and by the writer in 1921 and 1922. (2) When the rate of seeding was increased to compensate for wheat killed in treatment, treated grain outyielded untreated grain which contained 10.7 per cent of loose smut. (3) The yield from treated seed sown wet was not so high as that from treated seed sown dry. Freeman and Johnson (*4*) noted that where treatment was injurious, seed improved in vitality, in general, as the length of time after treatment increased. In the foregoing experiment the dry-sown seed was treated a week before the wet-sown seed, and it seems probable, therefore, that the higher yield of the dry-sown wheat may be attributable to a better field germination. The results shown in Table XVI indicate the desirability of making a soil germination test before sowing to determine the amount of seed killed by treatment and of increasing the rate of seeding to compensate for this amount.

It has been claimed that the modified hot-water treatment kills the small shriveled kernels in a seed lot and, as a result, wheat grown from treated seed produces more vigorous plants and grain of higher bushel weight. In order to determine first the effects of treatment on large and small kernels, three lots of machine-threshed Goens and one lot of machine-threshed Leap (C. I. 4823) wheat were used. One hundred of the largest and one hundred of the smallest kernels were selected from each lot and treated on December 5, 1922. A like number were selected and left untreated. The small kernels frequently were shriveled and distorted. The treated grain was allowed to dry a week and the untreated and treated kernels were sown on December 12, in soil in greenhouse flats. One month later, on January 12, 1923, germination counts were made. The results are presented in Table XVII.

TABLE XVII.—*Percentages of germination of large and small kernels of machine-threshed Goens and Leap wheat, untreated and treated by the modified hot-water method*

Lot No.	Variety	Percentage of germination					
		Large kernels			Small kernels		
		Untreated	Treated	Reduction due to treatment	Untreated	Treated	Reduction due to treatment
32	Goens	88	38	50	77	29	48
35	do	93	45	48	73	28	45
37	do	92	51	41	64	25	39
38	Leap	76	23	53	66	32	34
Average		87.3	39.3	48.0	70.0	28.5	41.5

Reference to Table XVII shows that reduction in germination due to treatment was 6.5 per cent greater for the large seeds. This probably was due to the fact that large kernels exposed more surface to threshing injury, sustained greater damage to the seed coats, and consequently suffered greater injury from treatment. Schellenberger (*11*) has noted that the threshing machine usually damages large kernels.

In order to determine the effects of treatment on the weight of grain, a test weight per bushel was taken of all of the wheat grown from the untreated and treated seed of five lots used in the rod-row yield experiments of 1922. The results are presented in Table XVIII.

TABLE XVIII.—*Bushel weight of wheat grown from five lots of untreated and of treated seed used in the yield experiments of 1922 (see Table XIV)*

Lot No.	Variety	C. I. No.	Bushel weight in pounds of wheat grown from—									
			Untreated seed sown in plats					Treated seed sown in plats				
			1	2	3	4	5	1	2	3	4	5
16	Red Chaff		55.25	55.00	54.75	55.00	55.00	56.25	56.50	54.25	54.50	54.50
17	do		54.00	56.00	52.75	53.00	53.50	54.75	57.00	53.50	53.75	53.50
19	do		56.00	56.00	55.00	55.75	53.75	55.50	55.00	54.75	55.50	53.25
26	Fulcaster	6162	51.50	51.75	51.00	51.00	50.50	51.25	51.50	52.00	52.50	50.50
27	Purplestraw	1915	55.00	54.50	56.25	56.50	55.75	55.00	55.00	55.75	56.00	55.75
	Average		54.20					54.31				

Table XVIII shows a difference in weight of grain in favor of wheat from treated seed but it amounts to less than 2 ounces per bushel.

DISCUSSION

The effects of the modified hot-water treatment on wheat, so far as determined, have been shown to be largely dependent on the conditions of seed coats and soil. The latter varies greatly and the physical condition of the seed coats is dependent on the combination of such variable factors as the kind of weather during the period from ripening to threshing of grain, adjustment and speed of cylinder in the threshing machine, size of the wheat kernel, and handling of grain after threshing. It is evident, therefore, that the action of treatment varies and can not be predicted. The effects of treatment on the germination of machine-threshed seed were severe. The average soil germination of 58 different lots of machine-threshed seed (Tables III and VII) representing 32 distinct varieties was 33.3 per cent less for the treated than for the untreated wheat. In none of the lots did the germination of the treated seed equal that of the untreated. Even when the seed coats were intact, the modified treatment measurably retarded germination although it reduced germination very little, if at all. There is some evidence which seems to indicate that the action of treatment on spaced plants may extend beyond the germination period and into the later stages of plant growth. With regard to the effects of treatment on yield it has been shown that under the conditions of the experiments reported herein, plants from treated seed did not outyield plants from untreated seed except when sown at a rate compensating for treatment injury and when the plants from untreated seed contained a relatively high percentage of loose smut.

SUMMARY

(1) The development in recent years of a number of central or community plants for seed-treatment in Indiana and Virginia has stimulated interest in the control of the loose smut in wheat by the modified hot-water method and in the effects of treatment on the germination, growth and yield of wheat.

(2) The method of germinating treated seed in moist blotters can not be used to determine accurately the effects of treatment on the germination of seed when sown in the soil.

(3) Other factors being equal, the effects of the modified hot-water treatment on the germination of wheat are governed by the physical condition of the seed coat.

(4) The modified hot-water treatment reduced the germination of wheat seed to zero or nearly zero when the seed coats were broken over the embryo. Seed not killed germinated abnormally.

(5) Reduction and abnormality in germination also occurred when seed coats were broken over the endosperm, but somewhat less severely. The degree of severity depended upon the location of the fracture.

(6) Seed with unbroken coats suffered a retardation in germination following treatment but there was little if any reduction in germination.

(7) Removal of the pericarp did not diminish the protective ability of an otherwise unbroken seed coat.

(8) Unbroken seed coats exerted their protective action during both the presoak and 10-minute baths of the modified hot-water treatment; the major portion during the 10-minute treatment.

(9) Increase in the duration of presoaking increased the amount of injury from the 10-minute treatment rapidly when the seed coats were broken, and relatively very slowly when the seed coats were unbroken.

(10) In the absence of any presoak period, the 10-minute treatment caused severe injury when seed coats were broken over the embryo.

(11) The rate of water absorption by the seed was more rapid when the seed coats were broken.

(12) Injury to seed coats is caused mainly in the process of threshing. All machine-threshed seed examined showed broken seed coats.

(13) Widely different weather conditions during the period between ripening and harvesting of seed had a marked effect on the amount of injury sustained by seed coats in the process of threshing and consequently in the amount of injury sustained by the seed from treatment.

(14) Small, shriveled kernels survived treatment as well or better than large, plump kernels from the same lot of machine-threshed seed.

(15) Injury and killing caused by treatment when the seed coats are broken may be due to coagulation of leucosin in the wheat embryo.

(16) Plants grown from treated seeds spaced in the rows were fewer in number and produced slightly fewer culms per plant than spaced plants from untreated seeds.

(17) Under less favorable soil conditions, the reduction in yield from treated seed was over four times the reduction in yield from untreated seed.

(18) The bushel weight of wheat grown from treated seed was not appreciably greater than that grown from untreated seed.

(19) Yield experiments were conducted for three years and a total of nine different lots was employed. In each of the three years wheat grown from untreated seed outyielded that grown from treated seed when the rate of seeding was 6 pecks per acre. In the third year, treated seed yielded better than untreated smutted seed when the former was sown dry and when the seeding rate was adjusted to compensate for wheat killed in treatment, as determined by a germination test in soil.

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PLATE 1

A.—Healthy head of Goens (Red Chaff) wheat in bloom and a head of the same variety affected with loose smut. The periods during which the healthy heads are in flower and spores are blowing from the diseased heads are coincident. Infection takes place intraseminally.

B.—At the close of the flowering period of the healthy heads. Practically all of the smut spores have been blown from the diseased heads.

A
B

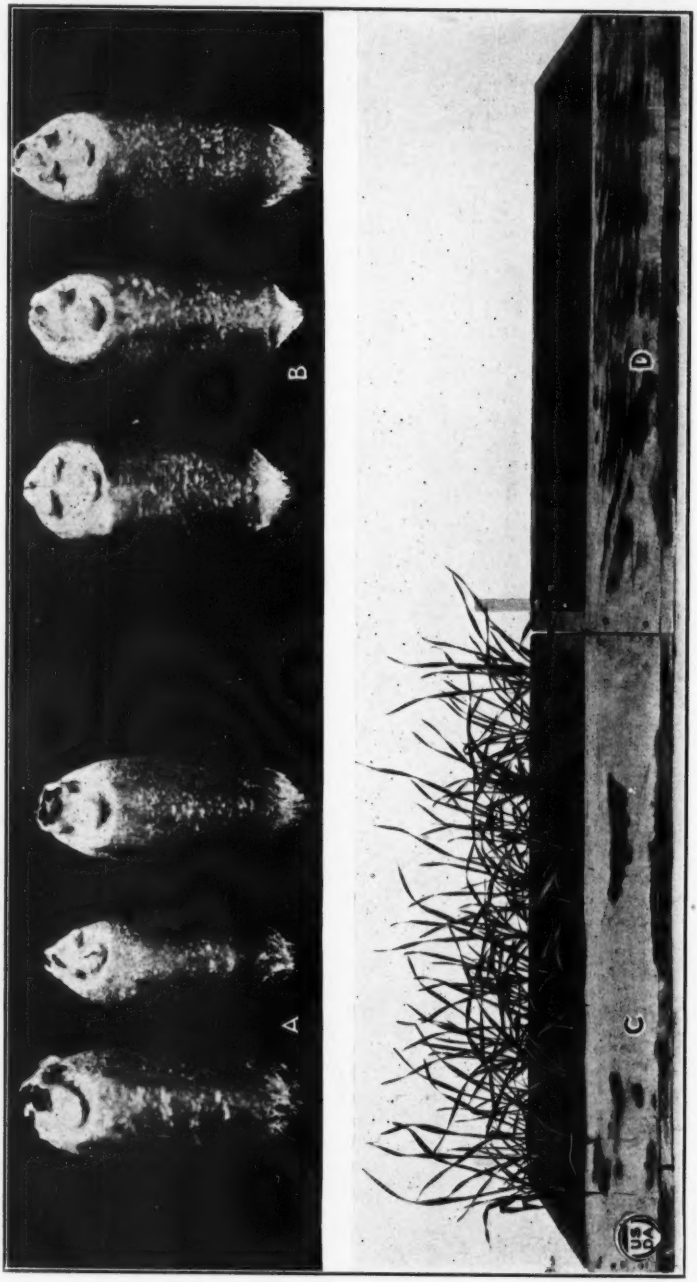
B
A
D
C

PLATE 2

Kernels of Goens (Red Chaff) wheat (A) and Minturki wheat (B) with seed coats broken over the embryo in threshing. (Photo. × 6.)

Effects of the modified hot-water treatment on seeds with coats unbroken and broken over the embryo.

C.—Fifty plants from 50 hand-threshed seeds of Poole wheat, the seed coats of which were slit over the embryo with a needle, but seeds not treated.

D.—Zero plants from 50 hand-threshed seeds of the same Poole wheat slit over the embryo with a needle and seeds treated.

PLATE 3

Some of the effects of the modified hot-water treatment on seeds with coats unbroken and broken over the endosperm only.

Five seedlings from hand-threshed treated kernels of China wheat. The four distorted seedlings (right) came from seeds the coats of which had been cut over the endosperm at the brush end before treatment. The tall seedling (left) came from a treated seed with coat unbroken.

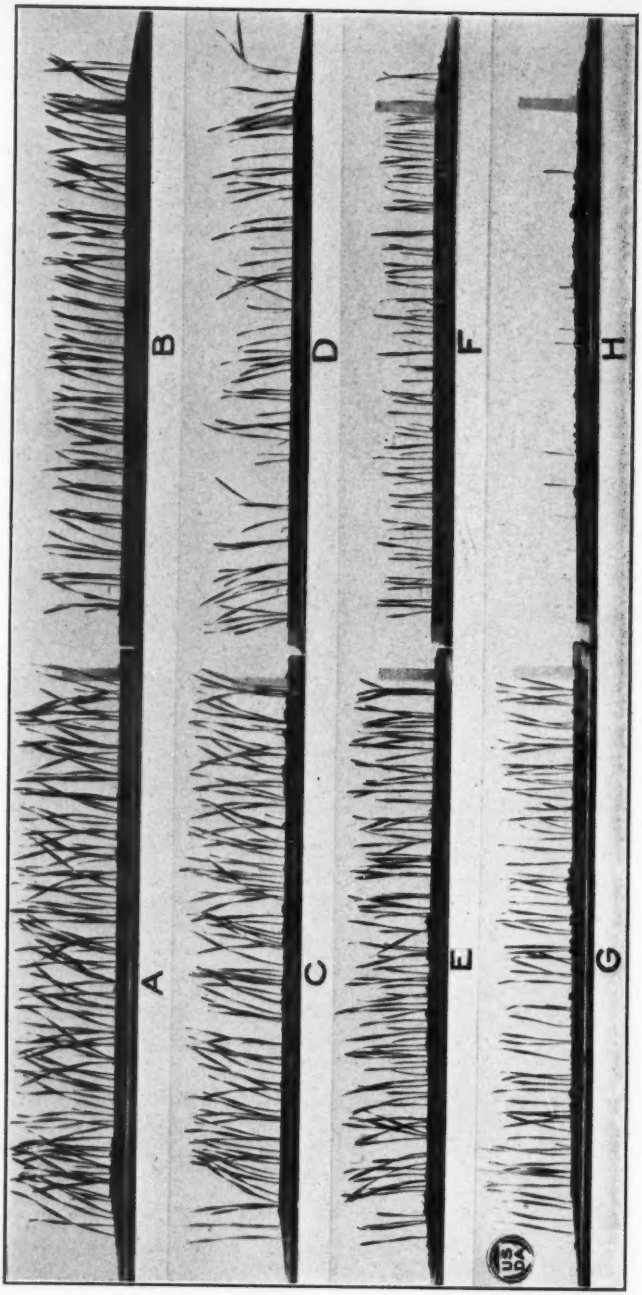
A
B
C
D
E
F
G
H
USDA

PLATE 4

Effects of the modified hot-water treatment on the germination and seedling development of Red May (Michigan Amber) wheat (seed lot *T* in Table VII) when threshed by hand and threshed by machine. One hundred seeds sown per flat.

A.—Hand-threshed seed, untreated. Germination 99 per cent.

B.—Hand-threshed seed, treated. Germination 100 per cent. Note retardation.

C.—Machine-threshed seed, untreated. Germination 99 per cent.

D.—Machine-threshed seed, treated. Germination 68 per cent when photographed, but final germination 77 per cent.

Effects of the modified hot-water treatment on the germination and seedling development of Dicklow wheat (seed lot *W* in Table VII) when threshed by hand and threshed by machine. One hundred seeds sown per flat.

E.—Hand-threshed seed, untreated. Germination 100 per cent.

F.—Hand-threshed seed, treated. Germination 97 per cent. Note retardation.

G.—Machine-threshed seed, untreated. Germination 84 per cent.

H.—Machine-threshed seed, treated. Germination 15 per cent when photographed, but final germination 34 per cent.

PLATE 5

Effects of hot-water treatment on germination of hand-threshed seed of the variety Haynes Bluestem (C. I. 2874), and on seedling development, as influenced by integrity of seed coat and length of presoak. (See Table IX.)

A.—Seed coats unbroken, seed not treated. Germination 96 per cent.

B.—Seed coats cut over the embryo, seed not presoaked before treatment for 10 minutes at 54° C. Germination 38 per cent.

C.—Seed coats cut over the embryo, seed presoaked for 1 hour before treatment for 10 minutes at 54° C. Germination 19 per cent.

D.—Seed coats cut over the embryo, seed presoaked for 2 hours before treatment for 10 minutes at 54° C. Germination 15 per cent.

E.—Seed coats cut over the embryo, seed presoaked for 4 hours before treatment for 10 minutes at 54° C. Germination 4 per cent.

F.—Seed coats unbroken, wheat presoaked for 10.5 hours before treatment for 10 minutes at 54° C. Germination 93 per cent.

A
B
C
D
E
F